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**Denitrification woodchip bioreactor two-phase column study: Evaluation of nitrate removal at various hydraulic retention times and effect of temperature on denitrification rates**

by

**Natasha Lynn Hoover**

A thesis submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of  
**MASTER OF SCIENCE**

Co-majors: Environmental Science; Sustainable Agriculture

Program of Study Committee:  
Alok Bhandari, co-Major Professor  
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Iowa State University  
Ames, Iowa  
2012

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## **Chapter 1 : General Introduction**

### **1.1 Introduction**

The transport of nitrate to surface waters is an increasing concern in United States and throughout the world. Causal linkages have been established between nutrient loading from the Mississippi River Basin watershed and the formation of the large hypoxic zone in the Gulf of Mexico near the mouth of the Mississippi River (Gulf Hypoxia Action Plan 2008). A quantitative environmental goal of a 30% nitrate load reduction by 2015 was established in 2001 (Mississippi River/Gulf of Mexico Watershed Nutrient Task Force 2001); however, implementation of best management practices to meet these goals were based largely on voluntary efforts of stakeholders within the watershed (Rabalais et al. 2002). A landuse model by McIsaac et al. (2001,2002) suggests a reduction of 12–14% in nitrogen load from agricultural lands will be required to meet the overall reduction goal. Surface applications of fertilizers lead to improved production yields, but there is concern that these same chemicals pose a risk to water quality. According to the USDA's 2007 Census of Agriculture, 86% of Iowa's total land area is utilized for agriculture. Commercial fertilizers, which can contribute nitrogen to waterbodies through leaching and runoff into local water systems, are applied to 61.8% of Iowa's 30,747,550 acres of farmland. (USDA, 2009). Reducing nitrogen export from the upper Midwest is a key to maintaining and improving the quality of local, national, and global water resources.

Although nitrogen fertilizers provide a measurable benefit to the agricultural industry, the increased nitrate exposure is potentially harmful to human health when consumed in drinking water, and detrimental to the aquatic ecosystems. Several health impairments have been linked to increased nitrate consumption in humans. The Environmental Protection

Agency imposed a maximum contaminant level on drinking water of  $10 \text{ mg L}^{-1}$  in response to an increased incidence of methemoglobinemia, or “blue baby syndrome”, attributed to formula prepared with well water containing high concentrations of  $\text{NO}_3\text{-N}$  (EPA, 1991). A link to increased methemoglobinemia in infants and nitrate intake was further documented in Moroccan study of drinking water with high nitrate concentrations (Sadeq et al., 2008). Additional health risks associated with high nitrate consumption include thyroid dysfunction, colon cancer, and ovarian cancer (Powlson et al., 2008).

The transport of nitrate into surface and marine waters also poses a risk to aquatic environments. Nitrate loading into surface waters can result in eutrophication, a condition of overgrowth of primary producers on the water’s surface. While an increase in photosynthetic growth may appear beneficial, this condition actually results in oxygen depletion of the water because the rate of decomposition (which consumes oxygen) also increases. The oxygen depleted water is no longer able to support the same level and diversity of aquatic life, as organisms must either migrate, adapt, or perish in response to insufficient dissolved oxygen levels (Diaz and Rosenberg, 2008; EPA, 2012).

The installation of tile drainage, a series of perforated pipes 2-6 feet below the soils surface, is commonly used to lower the water table, and drain excess water from fields. This tile drainage exacerbates the problem by providing a direct conduit from field to surface water bodies, resulting in high nitrate loads being transported from tile drained fields. This mode of transport is greatly reducing the natural denitrification and plant uptake of nitrates that would occur in a natural stream system, resulting in increased nitrate loading to surface waters (David et al., 2010; Sands, et al., 2008).



Denitrification woodchip bioreactors are a relatively new technology for edge of field removal of nitrates (Christianson et al., 2010a,b, Woli et al., 2010). These engineered denitrification systems provide an environment that supports the growth of denitrifying bacteria, and compensate for the reduced interaction of tile drainage with natural biological soil processes. Tile drainage is routed through the bioreactor, where the nitrates are removed via denitrification. In the simplest terms, a denitrifying woodchip bioreactor is a large pit filled with woodchips, and sealed with an overburden of soil.

These woodchip bioreactors exhibit a promising potential as a remediation tool for removing nitrates from outgoing tile drainage; however, bioreactors have shown varying degrees of success with nitrate removal (Woli et al., 2010). Correlation between environmental factors and bioreactor function are not well documented, and additional research is needed to quantify the impact of hydraulic and environmental factors such as temperature.

## **1.2 Goals and Objectives**

The overall goal of this study was to better understand the relationship between hydraulic retention time and temperature on denitrification in woodchip denitrification bioreactors. The objectives of this study were:

- To identify potential environmental ramifications with woodchip bioreactors by quantifying total organic carbon (TOC) release during initial start-up of the column bioreactors
- To compare nitrate removal at various hydraulic retention times

- To compare nitrate removal rates and efficiency at various influent nitrate concentrations at consistent HRTs
- To quantify bioreactor function (denitrification) at various influent nitrate concentrations under controlled temperature (20<sup>0</sup> C, 15<sup>0</sup> C, and 10<sup>0</sup> C) conditions at a target 12-hour HRT

### **1.3 Hypothesis**

The hypotheses for this study were:

- Total organic carbon release will decrease as soluble carbon and residues are washed from the system
- Increases in hydraulic retention time will result in increased nitrate removal (denitrification)
- Decreases in ambient and influent temperature will result in reduced rates of denitrification

### **1.4 Thesis organization**

This thesis will follow a traditional format, beginning with an overall introduction in chapter 1, followed by a review of relevant literature in chapter 2. Chapter 3 will detail the methods and materials of the study. The results and discussion will be presented in chapter 4, and the text will conclude with the conclusions and suggestions for future research in chapter 5.

## Chapter 2 : Literature Review

### 2.1 Impacts of nitrate on the environment and human health

Research continues to explore the link between high nitrate intake and health concerns. It has been suggested that several cancers, including intestinal and colon cancers, thyroid disease, and ovarian cancers are associated with high nitrate concentrations in drinking water. A well documented health concern associated with high nitrate intake by infants is methemoglobinemia. Methemoglobinemia, known as “blue baby syndrome”, is a condition in which the transport of oxygen throughout the body is inhibited, resulting in oxygen deficiency and potential death. Because the intestines of infants have a more neutral pH, bacteria which convert consumed nitrate into nitrite are able to survive. The nitrite that is produced interferes with the ability of hemoglobin in the blood to carry oxygen (Greer and Shannon, 2005). A survey conducted in the early 1950s found a possible link between infant illness and potential death due to methemoglobinemia and consumption of well water with nitrate concentrations of  $20 \text{ mg L}^{-1}$  or greater. The results of the survey, along with limited results from a controlled study on the effects of high nitrate intake by infants, prompted the Environmental Protection Agency (EPA) to set a maximum contamination level (MCL) of  $10 \text{ mg L}^{-1} \text{ NO}_3\text{-N}$  for drinking water. Research also suggests an increased occurrence of thyroid dysfunction in individuals exposed to high levels of nitrate in their drinking water (Manassarman et al., 2006).

In addition to the potential impacts of excess nitrate exposure to human health, nitrates have been documented to impact the health and diversity of aquatic ecosystems.

Aquatic life is impacted directly by increased exposure to nitrates, as well as by the resulting eutrophication and hypoxia. Local lakes and streams may experience eutrophication, a condition where photosynthetic algae is fertilized by the influx of nutrients. The result is the characteristic green water and mossy film caused by this algal overgrowth, or 'algal bloom'. The aquatic ecosystem is altered both chemically and physically, with the depletion of DO as the increased available biomass is decomposed, and with the decreased light penetration caused by the increased cloudiness. (Camargo and Alonso, 2006; Diaz and Rosenberg, 2008).

Hypoxia is defined as a shortage of dissolved oxygen within a water column, and is measured in terms of  $O_2$  concentrations below  $2 \text{ mg L}^{-1}$  or  $1.42 \text{ ml L}^{-1}$  (Levin et al., 2009). The Mississippi River/Gulf of Mexico Watershed Nutrient Task Force defines hypoxia more broadly, as dissolved oxygen concentrations of less than  $2\text{-}3 \text{ mg L}^{-1}$  (Mississippi River Basin Watershed Nutrient Task Force, 2010). There are multiple interacting factors contributing to the hypoxic zone of the Gulf of Mexico, including nutrient influx, specifically nitrates, from the Mississippi River Basin and the resulting explosion of phytoplankton growth in the coastal waters, increased oxygen consumption as phytoplankton biomass is decomposed, and stratification of the inflowing freshwater and more dense saline water of the Gulf. An influx of freshwater carrying dissolved nitrates into the Gulf of Mexico results in the fertilization of the coastal waters in which algal growth is otherwise limited by nitrate availability.

Nitrates in aquatic systems have a similar effect on some aquatic life, such as crayfish and minnows, as nitrates in drinking water have on infants. The nitrates interact with the oxygen carrying pigments (hemoglobin and hemocyanin) of aquatic organisms, interfering with the ability to carry oxygen (Scott and Crunkilton, 2000; Cheng et al., 2002). Research

suggests hypoxia is having negative impacts of reproductive health of specific fish species in the Gulf of Mexico (Thomas and Rahman, 2011, 2010, 2009; Hamlin et al., 2008). One such species, the Atlantic croaker, has shown declining egg viability and sperm production in fish collected from known hypoxic sites in the Gulf of Mexico. A decline in viable egg production of approximately 50% was observed in female Atlantic croakers from a hypoxic site (bottom DO:  $2.1 \text{ mg L}^{-1}$ ; water depth: 18 m) compared to specimens collected from the normoxic site (bottom DO:  $6 \text{ mg L}^{-1}$ ; water depth: 20 m) (Thomas and Rahman, 2009). A subsequent study demonstrated a 2-3 fold decline in sperm production in male Atlantic croaker collected from known hypoxic sites in the Gulf of Mexico compared to specimens collected from control sites documented to rarely experience hypoxic conditions (Thomas and Rahman, 2010). In addition, ovarian masculinization, as determined by an increased occurrence of male germ cells detected in the ovaries of Atlantic croakers, has been observed in specimens collected from hypoxic sites in the Gulf of Mexico (Thomas and Rahman, 2011).

Hypoxic areas, such as the Gulf of Mexico 'Dead Zone', experience reduced diversity and decreased population densities of aquatic species that are not tolerant of low dissolve oxygen (Diaz and Rosenberg, 2008). While the Atlantic Croaker is able to adjust, at least temporarily, to unfavorable dissolved oxygen conditions, other species implement other survival techniques. Sensitive organisms that are less mobile will experience declining populations as the organisms die-off when conditions become unfavorable. Mobile organisms, such as brown shrimp, will migrate to seek environmental conditions that are favorable to growth and survival. The population distribution and densities of brown shrimp

(*Farfantepenaeus aztecus*) in the Gulf of Mexico are impacted by the large hypoxic zone off the coast of Louisiana. Due to the low DO of the shrimp's preferred habitat, the shrimp are populating in greater densities the more shallow inshore waters and deeper offshore waters outside of the hypoxic zone (Craig et al., 2005). Shifts in population distribution and density may have financial impacts on the fishing industry in the Gulf of Mexico, as vessels are required to travel further offshore for harvests.

## **2.2 Tile Drainage accelerates nitrogen transport to waters**

Natural wetland soils have been drained to expand and improve agricultural production throughout the Midwestern United States. Over 80-87% of soils in the United States that have been drained were drained for agricultural purposes (McCorvie and Lant, 1993). The largest extent of these drained wetland soils have been in the United States Midwest, specifically in the Cornbelt (Illinois, Indiana, Iowa, and Ohio). An estimated 90-97% of original wetlands in the United States' Cornbelt have been artificially drained for agricultural purposes (McCorvie and Lant, 1993).

The majority of nitrate loading to the Gulf of Mexico is attributed to the Upper Mississippi River Basin, with over 75 percent of the annual estimated load of  $\text{NO}_3 + \text{NO}_2$  -N transported to the Gulf of Mexico originating from the MRB (USGS, 2011). Despite evidence of the impacts on nitrate loading on aquatic and human health, there has been an overall increase in loading from the MRB to the Gulf of Mexico over the past decade (USGS, 2011).

Policy has had a major impact on the adoption of agricultural tile drainage. The United States has a long history of policies supporting agricultural tile drainage. In the past,

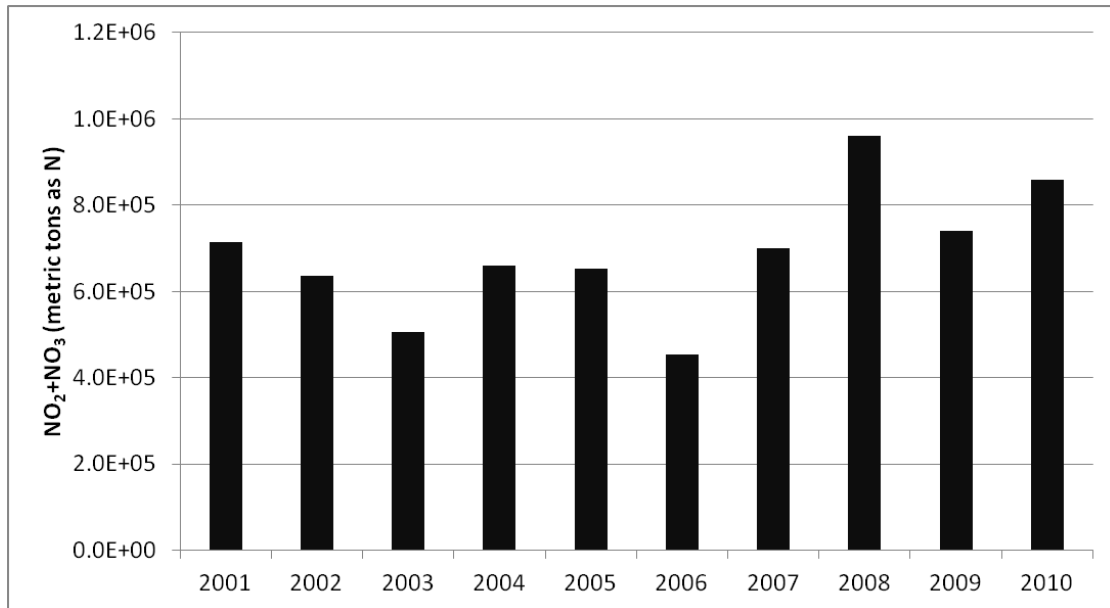
wetlands were considered unproductive land that served as a potential breeding ground for infectious viruses and other diseases. The Swamplands Act of 1850 set aside acreages of wetlands to be drained, and enhanced to support agricultural development. The United States Department of Agriculture (USDA) provided cost-share benefits for wetland drainage through the mid-1950s, and technical assistance for wetland drainage was available through the Soil and Conservation Service (SCS) until 1972 (Nelson, 1986). The major crops benefiting from agricultural drainage, corn and soybeans, are also federally subsidized, further incentivizing the installation of tile drainage. (McCorvie and Lant, 1993).

Tile drainage has contributed to increases in agricultural production, increasing the amount of arable land and allowing farmers earlier access to fields that would otherwise be saturated. (Busman and Sands, 2002). However, tile drainage has also been implicated in negative environmental impacts. A major concern related to tile drainage is an increase in pollutant loading of contaminants dissolved or otherwise transported in tile drainage flow. Estimated and measured tile drainage  $\text{NO}_3\text{-N}$  concentrations are variable. Sands et al.(2008) reported tile drainage nitrate concentrations of 10.2 to 13.1 mg/L during soybean rotation years, and 13.3mg/L to 20.4 mg/L during corn rotation years.

### **2.3 Nitrate loading attributed to the Mississippi River Basin**

The transport of nitrates from the Mississippi River Basin (MRB) have been indicated as the primary contributor (Table 2.1) to a large hypoxic zone known as the ‘Dead Zone’, in the coastal waters of the Gulf of Mexico (USGS, 2011). Over the past decade, the MRB has contributed to an average of 76% of the total nitrate loading to the Gulf of Mexico (Table 2.1). Despite evidence of the impacts of nitrate loading on human and aquatic health, there

has been an overall increase in loading from the MRB to the Gulf of Mexico over the past decade (Figure 2.1).



**Figure 2.1 NO<sub>2</sub> + NO<sub>3</sub> estimated annual load from the MRB to the Gulf of Mexico from 2001 to 2010. Data source: USGS**

The yearly nitrate load contributions to the Gulf of Mexico from the Mississippi River Basin are presented in Table 2.1. Estimates were calculated from the USGS monthly nutrient flux and concurrent streamflow data (USGS, 2011). Table 2.1 shows no apparent trend with nitrate load contributions and the estimated yearly flow volume from the Mississippi River Basin into the Gulf of Mexico. Total nitrate (NO<sub>2</sub> + NO<sub>3</sub>-N) load does not appear to directly correlate to yearly flow values.



**Table 2.1 MRB load contribution to the Gulf of Mexico**

| Mississippi River Basin Load Contribution to Gulf of Mexico |                   |   |         |        |
|---|-------------------|---|---------|--------|
| Year  | Yearly Flow       | NO <sub>2</sub> +NO <sub>3</sub> (Metric Tons as N) |         |        |
|   | m <sup>3</sup> /s | Gulf of Mexico                                      | MRB     | % Load |
| 2001  | 189,410           | 898,350   | 713,180 | 79%    |
| 2002  | 237,120           | 817,750   | 635,650 | 78%    |
| 2003  | 220,600           | 671,650   | 504,900 | 75%    |
| 2004  | 233,070           | 862,300   | 658,750 | 76%    |
| 2005  | 239,470           | 869,300   | 651,585 | 75%    |
| 2006  | 138,850           | 611,300   | 453,500 | 74%    |
| 2007  | 209,620           | 899,800   | 700,300 | 78%    |
| 2008  | 287,750           | 1,243,100   | 960,400 | 77%    |
| 2009  | 236,410           | 989,150   | 740,650 | 75%    |
| 2010  | 298,200           | 1,135,800   | 859,150 | 76%    |

While the lowest annual flow value corresponds to the lowest total nitrate load attributed to the MRB, the same correlation is not evident with most flow values and total nitrate load. The seventh highest total nitrate load was observed with the second lowest yearly flow from the Mississippi River Basin.

### **2.3 Factors determining rates of nitrate removal**

Respiratory denitrification is the main mechanism for dissimilatory nitrate reduction in the soil, and the primary mechanism for nitrate removal in engineered denitrification systems (Sylvia et al., 2005; Schipper et al, 2010). Denitrification requires four essential elements to occur; an active community of denitrifying bacteria, oxygen depleted conditions, a readily available carbon source, and the presence of NO<sub>3</sub>-N as an alternative electron acceptor. Rates of denitrification are further influenced by temperature and pH (Seitzinger et al., 2006).

Denitrifying bacteria, also referred to as denitrifiers, represent an extensive suite of bacteria abundant in soil, comprising 0.1% to 5% of the total population of bacteria in the soil (Sylvia et al., 2005). Denitrifier populations in the soil have been measured at populations ranging from  $10^3 \text{ g}^{-1}$  to populations exceeding  $10^7 \text{ g}^{-1}$  in surface soil, and  $10^6 \text{ g}^{-1}$  in subsurface soil (Murray et al., 1995; Moorman et al., 2010). With several species of bacteria capable of denitrification, denitrifiers are classified as a group by physiological characteristics. Illustrating the diversity of denitrifying bacteria, the group includes members of the following genera: *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Chromobacterium*, *Corynebacterium*, *Flavobacterium*, *Hypomicrobium*, *Moraaxella*, *Paracoccus*, *Propionibacterium*, *Pseudomonas*, *Thizobium*, *Rhodopseudomonas*, *Spirillum*, and *Vibrio* (Sylvia et al., 2005). *Pseudomonas* is the most common and widely distributed of all denitrifying bacteria (Metcalf, 2003; Sylvia et al., 2005).

The complexity of the denitrifying bacteria community makes quantification of organisms difficult. Cultivation techniques, such as most probably number (MPN), may underestimate denitrifying bacteria populations. Michotey et al. (2000) demonstrated that molecular techniques for quantification (PCR) result in denitrifying bacteria counts of 3 to 10 orders of magnitude greater than traditional cultivation techniques. Additionally, the molecular techniques used in the Michotey study did not account for additional denitrifying bacteria present with the copper reductase enzyme, which were included in counts using cultivation methods. There are several methods that can be used to determine potential

denitrification rates, thus extrapolating the relative denitrifying community populations and dynamics, and bypassing the challenges of direct quantification of bacterial populations.

Generalized bacterial growth rates, which have been quantified as an exponential growth curve within a maximum carrying capacity of the specific environment, provide an approximation of denitrifying bacteria dynamics. The most common type of cell division (reproduction) in bacteria is binary fission, or the splitting of one cell into two cells by the formation of a partitioning membrane (Sylvia et al., 2005). Three phases of the bacterial growth curve have been successfully modeled as (1) the lag phase, or a period of acclimation (2) the exponential growth phase, or a period of successive cell doubling, and (3) the stationary phase, or no net growth (Buchanan et al., 1997). The lag phase is a brief period of zero, or very slow growth, as organisms adjust to environmental conditions (Sylvia et al., 2005). Once the bacteria have acclimated to the environment, an exponential growth rate is observed until the environment has reached its maximum carrying capacity. The period of exponential growth is characterized by a doubling of bacterial cell mass over a constant interval of time (Sylvia et al., 2005). Environmental conditions rarely provide the ideal conditions allowing for exponential growth. A deficiency in any of the factors required for denitrifier growth and denitrification can inhibit the exponential growth phase. As nutrients become limiting, and toxins accumulate throughout the growth phase, the rate of growth eventually slows, leading into the stationary phase. During the stationary phase, death rate is equivalent to the rate of growth, and biomass remains relatively constant. Additional research into bacterial growth and death dynamics suggests the occurrence of a death phase.

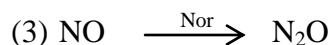
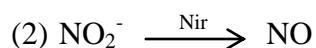
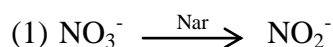
Populations of bacteria appear to be programmed for a specific lifespan, and organisms of the same cohort are documented to experience group death (Vasile and Graham, 2010).

Both microbial biomass and the quantity of soluble carbon determine the potential rate of denitrification (Blagodatsky and Richter, 1998; Blagodatsky et al., 2011). Since bacterial denitrification is a respiratory process, an electron donor source is required. Early research of denitrification kinetics suggest a carbon to  $\text{NO}_3\text{-N}$  ratio of 3 to 1 for complete nitrate removal in soil under conditions favorable for denitrification (Shah and Coulman, 1978). Additional research suggests a smaller C: N ratio may be required for complete denitrification. The carbon requirements as a C:N ratio with nitrate-N range between 0.93 and 1.32 for various carbon sources (Mateju, 1992). Labile carbon required for denitrification has been estimated at 1.28 moles  $\text{CH}_2\text{O}$  per mole  $\text{NO}_3$  removed, or approximately 1.07 g C per 1 g N (Robertson et al., 2008). When DO is present, the carbon requirements for denitrification are greater (Mateju, 1992).

In addition to the presence of an active community of denitrifiers and microbial available carbon, the environment must be depleted of oxygen for nitrate removal to occur. Oxygen is the preferred electron acceptor for cellular respiration of bacteria capable of denitrification, and will be used if available (Metcalf and Eddy, 2003). Denitrifying bacteria are facultative anaerobes, which can adapt to an environment that is depleted of oxygen to utilize nitrate as a terminal electron acceptor. As dissolved oxygen concentrations decline to levels below  $2 \text{ mg L}^{-1}$ , the environment is considered anoxic (oxygen depleted), and can support denitrification. With reduced oxygen availability, denitrifying bacteria begin utilizing nitrate.

Denitrification activity has been observed over a range of DO levels, with nitrate removal often attributed to denitrification in anaerobic microsite. As DO levels decline, denitrifying bacteria are able to utilize  $\text{NO}_3\text{-N}$  for respiration.

Denitrification is the reduction of  $\text{NO}_3$  to  $\text{N}_2$ , an inert gas and the main component of the Earth's atmosphere. Denitrification is a four-phase process, driven by the enzymatic activity of nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos). Nar facilitates the first step of denitrification, generating energy transporting adenosine triphosphate (ATP) as it catalyzes the reduction of nitrate to nitrite. Anaerobic conditions are required for Nar to function as the catalyst for this first step. The nitrite is further reduced to nitric oxide with Nir as a catalyst. Nitric oxide is then converted to nitrous oxide in the third step of denitrification. The Nor enzyme is the catalyst for this phase of denitrification. The final step in denitrification is the reduction of nitrous oxide to  $\text{N}_2$  gas, which is catalyzed by Nos (Sylvia et al., 2005). The equations representing the four phases of denitrification are:



The enzymes involved in denitrification are inhibited to varying degrees by the presence of DO, resulting in increased concentrations of denitrification intermediates determined by DO inhibition.

While the kinetics of denitrification continue to be researched, several studies suggest denitrification follows, at least in part, first-order decay rates. Since denitrification is an enzymatic driven process, research suggests that denitrification follows the Michaelis-Menten model for reaction rates (Messer and Brezonik, 1984). Michaelis-Menten kinetics take into consideration the availability and concentration of substrates involved in enzymatic reactions, such as denitrification (Sylvia et al., 2005). At low substrate availability, in this case nitrate, the decay rate follows a zero-order reaction rate; however, the majority of simple models consider denitrification a first order decay process (Heinen, 2006). Denitrification rates at low concentration or nitrate availability are independent of concentration. In a lab-scale woodchip bioreactor study with influent nitrate concentrations, Chun et al. (2010) determined nitrate removal to more closely follow a first-order reaction than a zero-order reaction. An additional substrate requirement of denitrification is microbial available carbon.

## **2.4 Variability in methods of denitrification measurement**

Methods and units of denitrification differ between studies, making direct comparisons of nitrate load removal and efficiency difficult. Methods for measurement include: measurement of enzymatic activity and byproducts, such as  $\text{N}_2\text{O}$ ; most probable number (MPN) techniques to quantify denitrifying bacteria and estimate denitrification potential; and mass balance analysis to determine actual  $\text{NO}_3\text{-N}$  actual denitrification as load removal (Warneke et al., 2011a,b,c). Actual denitrification is measured in units determined by the point or method of measurement. Accepted units of measurement include (Heinen, 2005):

- $\text{g N kg}^{-1} \text{ d}^{-1}$  when denitrification is based on the dry weight of the medium (soil or woodchips)
- $\text{g N m}^{-3} \text{ d}^{-1}$  when measurement based on the volume of the medium or container of interest
- $\text{kg N ha}^{-1} \text{ d}^{-1}$  when estimates refer to a specified layer of soil, in which case the soil layer thickness must also be reported
- $\text{mg N L}^{-1} \text{ d}^{-1}$  when denitrification refers to the loss of nitrate from solution

Nitrate removal in denitrification woodchip reactors and similar engineered systems for denitrification, such as denitrification beds and denitrification walls, are often reported as  $\text{g N m}^{-3} \text{ d}^{-1}$  or  $\text{g N kg}^{-1} \text{ d}^{-1}$ . Studies specific to smaller-scaled (pilot-scale and lab-scale) engineered systems for denitrification tend to report nitrate removal as  $\text{g N m}^{-3} \text{ d}^{-1}$  (Robertson et al., 2005; Schipper et al., 2010; Moorman et al., 2010). Moorman et al. reported nitrate removal in a denitrification woodchip bioreactor column study as  $\text{g N m}^{-3} \text{ d}^{-1}$ , which normalized for both bioreactor/woodchip volume and time.

## 2.4 Denitrification Woodchip Bioreactors

Microbial denitrification has been shown to be the primary mechanism for nitrate removal in denitrifying woodchip bioreactors (Robertson et al., 2000; Greenan et al., 2006; Greenan et al., 2009; Robertson, 2010; Schipper et al., 2010; Moorman et al., 2010; Warneke et al., 2011). Denitrification woodchip bioreactors are a promising technology for edge of field removal of nitrates from tile drainage, intercepting and treating drainage before it is released to surface waters. Research continues into the benefits of and potential negative

environmental impacts of bioreactors. Studies continue to explore the varying nitrate removal efficiencies in denitrification woodchip bioreactors, and the contributing factors involved. In addition, research has focused on potential negative impacts of woodchip bioreactors, such as greenhouse gas (GHG) emissions and excessive carbon loading into streams and waterbodies (Bergaust et al., 2010; Elgood et al., 2010; Misiti et al., 2011).

A variety of carbon substrates have been used in denitrifying woodchip bioreactors, including corn cobs, a variety of woodchips (hardwoods and softwoods), and sawdust (Warneke et al., 2011). Carbon substrates have been evaluated for nitrate removal efficiency and bioreactor longevity (decomposition). In previous studies, available carbon decreased during the first year of bioreactor operation, and then remained relatively constant (Schipper and Vojvodic-Vucovic, 2001). As microbial available carbon decreases, denitrifying bacterial populations decreased proportionally (Long et al., 2011). Research of denitrification potential in wetlands suggests a correlation between soluble carbon and denitrification rates (Gale et al., 1993). A study by Lin et al. (2007), which evaluated the denitrification rates within soils with differing amounts of extractable organic carbon, suggested a relationship between available carbon and denitrification. Sites with higher concentrations of extractable carbon generally exhibited higher denitrification rates than the sites with lower carbon. The highest measured extractable carbon concentrations were  $181 \pm 38 \mu\text{g C/g}$ , with a denitrification rate of  $15.02 \pm 1.87 \mu\text{g N}_2\text{O-N/g/h}$ . The soil with the lowest extractable organic carbon measurement,  $38 \pm 6 \mu\text{g C/g}$ , had a denitrification rate of  $0.11 \pm 0.20 \mu\text{g N}_2\text{O-N/g/h}$ . Statistical analysis suggested association with extractable carbon, but not a statistically significant fit with total carbon.



Under controlled conditions with constant nutrient availability, temperature, pH, and denitrifier population, nitrate removal follows an exponential decay curve (Abblebloom et al., 2010). Denitrifying woodchip bioreactors are not ideal systems, and therefore have dynamic conditions, with potential fluctuations in denitrifier community size, nutrient and labile carbon availability, temperature and pH. A lab-scale study by Chun et al.(2010) suggests stable biofilms are formed during periods of low flow rates (higher HRT), and subsequently washed away during high flow rates (low HRTs), directly resulting in lower denitrifying bacteria populations within the bioreactors. In addition, the more microbially available carbon particles may be washed away during high flow rates, reducing the available food source for the denitrifying bacteria.

Incomplete denitrification can result in increased release of  $\text{N}_2\text{O}$ , a potent greenhouse gas (Kornaros, 1996; Sylvia, 2005). A bioreactor study by Moorman et al. suggests nitrous oxide emissions in bioreactors do not exceed the emissions observed from tile-drainage when bioreactors are absent (Moorman et al., 2010). Variation in nitrate removal between studies of denitrification bed reactors is predominantly attributed to the operating temperatures and/or the influent nitrate concentrations (Schipper et al., 2010). The vast majority of nitrate removal in denitrifying woodchip bioreactors is contributed to heterotrophic bacterial nitrate removal (Warneke et al., 2011). Denitrification woodchip bioreactors are designed to create an environment favorable to denitrifying bacteria, supporting dissimilatory nitrate reduction (microbial activity).

Lab-scale and pilot-scale reactors are used to simulate full-scale reactors under controlled conditions, allowing for manipulation of parameters of interest, and the evaluation

of the nitrate removal response within the bioreactor. Lab-scale systems can be used to collect experimental data and supplement observed full-scale observations for verification of model accuracy, allowing for replicates of the same treatment or various treatments to be studied in unison. Lab-scale reactors have been used to study the treatment of wastewater, estimating such characteristics as the emission of  $\text{N}_2\text{O}$  from wastewater treatment (Hu et al., 2010). Studies have also looked at the use of various substrates or carbon sources to reactor efficiency. Pilot-scale studies involve a scaled-down model of the full-scale bioreactor.

Both lab and pilot-scale studies specific to denitrifying woodchip bioreactors have focused on the effects of HRT on denitrification, and denitrification efficiency with various carbon sources, decomposition and hydraulic characteristics of various carbonaceous and inert packing materials (Cameron and Schipper, 2010, 2011, Christianson et al., 2012, 2011, 2010). Several studies have also centered on denitrification bioreactor longevity and woodchip loss (Schipper et al., 2011).

## **2.5 Engineered Systems for Denitrification**

Engineered denitrification systems have been designed for treatment of drinking water, wastewater and surface water. Wastewater treatment systems are designed for hydraulic retention times of several days (Healy et al., 2006). Subsurface flow constructed wetlands are engineered systems that also utilize woodchips as a carbon source to support denitrification. Subsurface flow wetlands are classified with either vertical or horizontal flow patterns through the substrate. These systems are assumed to behave as plug-flow reactors (Saeed and Sun, 2011). First order kinetics, solely dependent on pollutant concentration and flow rate,

are generally assumed for biological degradation of pollutants in engineered systems designed for denitrification (Saeed and Sun, 2011). Research continues to be conducted to determine models that more accurately represent the dynamic characteristics and interactions that are actualized in these systems such as substrate concentrations (Saeed and Sun, 2011).

Lab-scale column denitrification woodchip bioreactors are designed as packed-bed reactors, which are a type of plug-flow reactor. Packed-bed reactors are filled with a packing material and solution is routed through the reactor as either upflow or downflow current. The reactors have little to no mixing of solute within the reactor. In a true plug flow reactor, all of the particles entering the reactor take the same amount of time to exit the reactor (Metcalf, 2003). The particles pass through the reactor in the sequence in which they entered, and theoretical retention time is equal to actual retention time (Metcalf and Eddy, 2003).

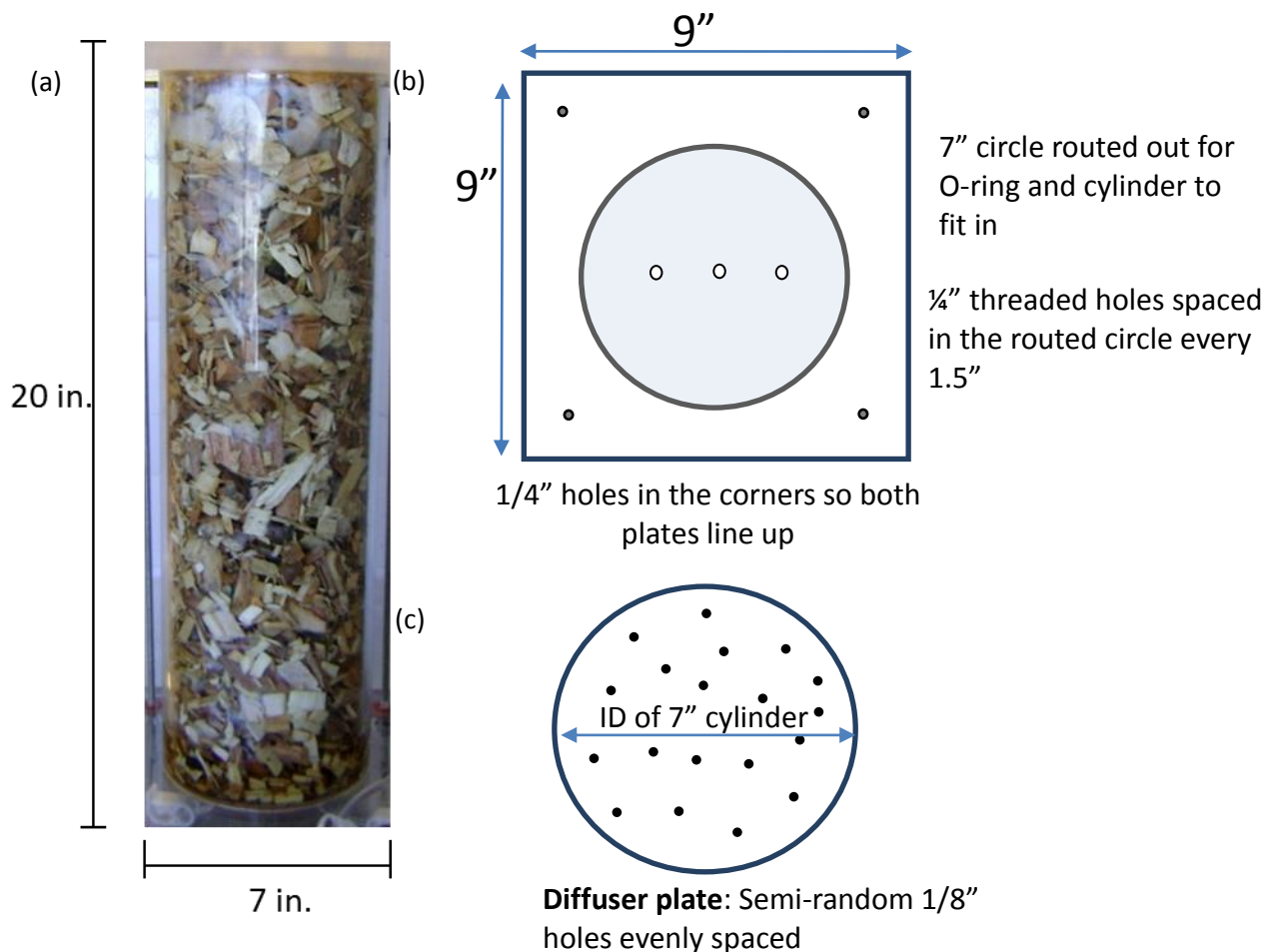
## **Chapter 3 : Methods and materials**

### **3.1 Introduction**

A series of triplicate column experiments were conducted to evaluate denitrification rates and efficiency under various controlled conditions in woodchip denitrification bioreactors. The first phase of the study was conducted at room temperature, and focused on denitrification woodchip bioreactor nitrate removal response with changes to hydraulic retention times and influent concentrations. Hydraulic retention time (HRT) was manipulated to compare removal in the denitrification bioreactors at target retention times of 2-hours, 4-hours, 8-hours, 12-hours, 18-hours, and 24-hours (achieved HRTs varied, and will be discussed in chapter 4). In addition to manipulation of hydraulic retention time, influent concentrations were adjusted at the 12-hour HRT. Three target influent concentrations were evaluated at the 12-hour HRT, 10 mg/L, 30 mg/L, and 50 mg/L (achieved influent concentrations varied, and will be discussed in detail in chapter 4).

The denitrification woodchip bioreactor columns were emptied and repacked with fresh woodchips after Phase 1, then moved to a temperature controlled chamber in the National Lab for Agriculture and the Environment for Phase 2 of the study. During Phase 2, HRT was maintained at a target of 12-hours. Denitrification response to ambient temperatures of 10<sup>0</sup>, 15<sup>0</sup>, and 20<sup>0</sup> C were monitored. Influent nitrate concentrations were also adjusted while the temperature was maintained at 20<sup>0</sup>C.

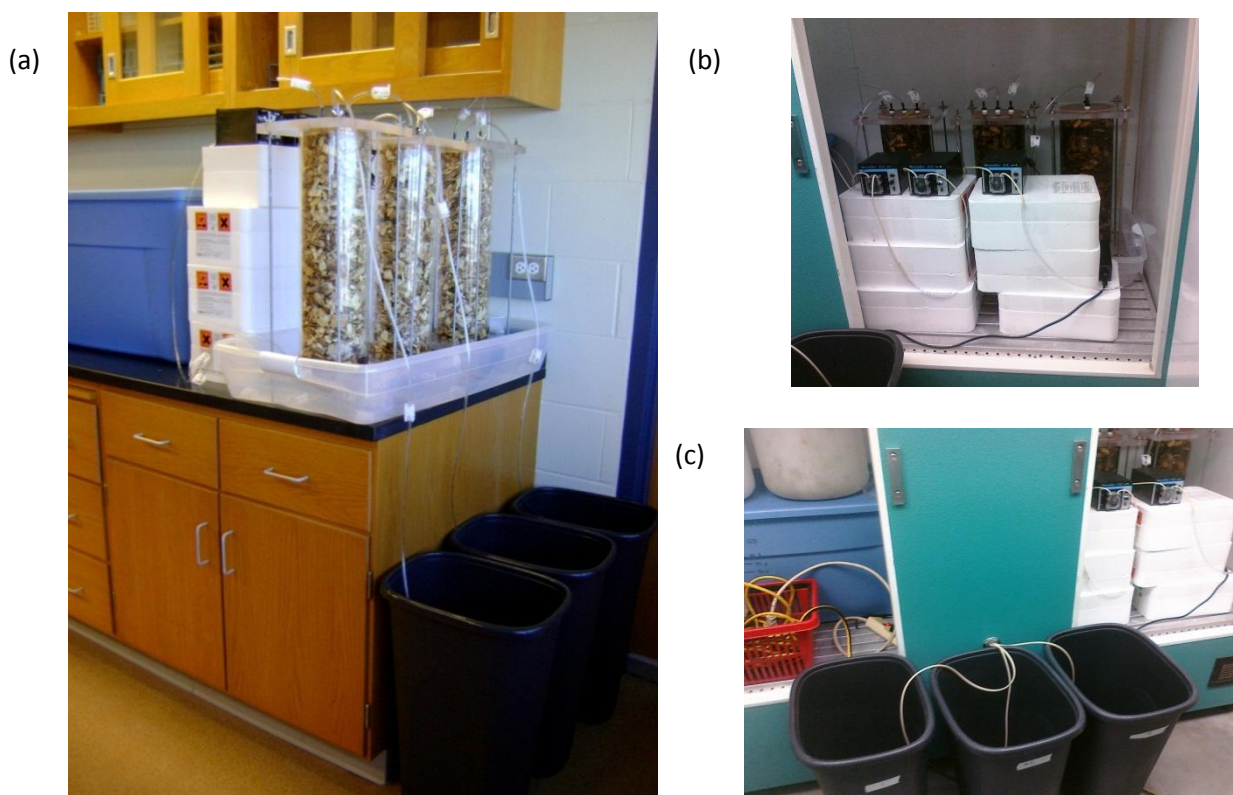
### 3.2 Denitrification bioreactor column design



**Figure 3.1 A photograph and schematic drawings of the woodchip denitrification bioreactor components. (a) photograph of a wood packed acrylic column (b) schematic of columns bioreactor end-plate (c) schematic of diffuser column bioreactor. The schematic images are modified from work done by L. Christianson.**

A triplicate denitrification woodchip bioreactor column study was conducted using three identically designed clear acrylic columns. Each column measured approximately 20 in. (50.8 cm) in height, with a diameter of 7 in (17.8cm). Perforated acrylic plates were fit at each end of the columns to diffuse the flow of fluid into the columns. After the columns were packed with woodchips, a rubber gasket was inserted between the column construction and

end-plates to create a water-tight seal. A threaded  $\frac{1}{4}$ " steel rod was inserted through the  $\frac{1}{4}$ " holes in the corners of the end plates of each column, with wingnuts at each end to secure and tighten the columns. Ports were located at the ends of each column, with tubing attached to allow for inflow and outflow of the synthetic tile solution.



**Figure 3.2 Denitrification woodchip bioreactor column experiment set-up. (a) Set-up of denitrification woodchip bioreactor columns for room temperature phase of the study; (b) and (c) show the columns set-up in a temperature controlled chamber.**

Figure 3.2 shows the triplicate column study layout for the room temperature phase (a) and the controlled temperature phase (b and c) of the study. During the room temperature phase of the study, the columns and containers were stored at room temperature. The large

influent container, columns, and pumps rested on top of the laboratory bench. The pumps were elevated on Styrofoam cubes to increase ease of access to pump controls and reduce the potential influence of a difference in hydraulic head on achieved flow rates. During the temperature controlled phase of the study, the chamber in which the columns were housed was not large enough for the entire column set-up. Only the influent tank and solution, the three pumps, and the three columns were kept in the temperature controlled chamber. The large effluent containers were positioned outside of the chamber, with the effluent tubing for each column routed through a small port in the wall.

The exact column volumes were measured by filling the columns with DI water, then measuring the mass of water drained from the columns with a K-Scale brand portable field digital scale. This scale had a maximum measurement capacity of approximately 31.75 kg, and displayed measurements to the thousandths kg. Due to the age and condition of the scale (this scale is no longer in production), accuracy of measurements were considered to the hundredths kg. The woodchip packed columns were drained three times throughout the course of the first phase of the study, and the volumes of drained solution were measured to calculate a mean pore volume for each column, assuming a solution density of  $1.00\text{g cm}^{-3}$  at room temperature, and therefore a conversion of 1 L per kg mass.

### **3.3 Woodchip characteristics**

Standard hardwood landscape woodchips from Golden Valley Hardscapes, located in Story City, Iowa, were used to pack the columns. The woodchips were packed into the columns and tamped intermittently to reduce void space using a steel rod. The packed columns were weighed at the beginning of the study to determine a preliminary pore volume

value, and were subsequently drained completely three times throughout the course of the study. The mean value for the initial pore volume based on weight and the drained volumes of solution in each column were used as the pore volume for each column.

The moisture content of the woodchips at the time the columns were packed was determined with measurements of mass loss due to drying of representative samples of woodchips. Three pans of additional woodchips were oven-dried at 70°C to determine the estimated moisture content of the woodchips at the time the columns were packed. The additional woodchips were dried until a change in mass was no longer detected (2-5 days), and the final mass was measured and recorded after the woodchips cooled to room temperature. The moisture content was reported as the percentage of water mass lost per dry mass of woodchips at equilibrium.

Gravitational porosity was calculated as the ratio of the volume of solution drained to the total bioreactor volume of each column. The secondary porosity, or porosity internal to the woodchips, was calculated for columns 1 and 3 were calculated at the end of the room temperature phase of the study. The woodchips from column 2 were not weighed before drying, so secondary porosity was assumed to be the mean secondary porosity for columns 1 and 3. The difference in the saturated weight of the woodchips from each column and the measured oven-dry weight of the woodchips at equilibrium was divided by the total volume of each column. The total porosity for columns 1 and 3 was calculated as the sum of the gravitational and secondary porosity. The total porosity for column 2 is derived using the mean secondary porosity for columns 1 and 3, and the gravitational porosity for column 2.



Moisture content of the woodchips was estimated using the measured mass loss of the sample woodchips to drying.

The bulk density was calculated based on the packed density. The moist density of the woodchips was calculated using the weight of the fresh woodchips packed into each column and the measured volumes. Additional woodchips from the same source batch were oven dried to determine a ratio to calculate the dry mass of woodchips used in this experiment. The ratio of dry weight to moist weight was then used to calculate the oven-dry weight at equilibrium (air temperature) of the woodchips in each column at the time the columns were packed.

Measured woodchip loss was calculated using a mass balance approach. The dry weight of the woodchips were estimated from the measured fresh weight of the woodchips when the columns were initially packed. The woodchips were then dried and re-weighed at the conclusion of the study. The measured dry weight of the woodchips at the end of the study were subtracted from the estimated dry weight of the woodchips at the start of the study to determine the mass loss of woodchips in each column over the course of the study.

### **3.4 Temperature coefficient**

The relationship between rate of nitrate removal and temperature was evaluating using a temperature coefficient. The temperature coefficient, or  $Q_{10}$ , is defined as the factor by which a rate of reaction increases with an increase in temperature of 10°C (Anderson and Janssens, 2006). Temperature Coefficient equation:

$$Q_{10} = (R_2/R_1)^{(10/\Delta t)}$$

Where  $R_2$  is the removal rate at  $t_2$ , and  $R_1$  is the removal rate at  $t_1$ ,  $\Delta t = t_2 - t_1$ ;  $t_2 > t_1$ . The  $Q_{10}$  factor was used to approximate changes in denitrification with increased temperature, but may over-simplify the complex interactions involved with temperature change and denitrification.

### **3.5 Flow Rate**

Target HRTs were achieved by controlling and manipulating pump speed and tubing size. Three Masterflex C/L 77122-22 variable speed peristaltic pumps were used to provide up-flow to individual columns. Tygon 0.89 mm ID tubing was used in the pumps to reach target flow rates corresponding to 18 and 24-hour hydraulic retention times. Assorted Tygon, Tygon LFL, and Pharmed BT tubing were used to achieve the higher flow rates, corresponding to the 12-hour and lower HRTs. Tubing wear affected flow rate, and contributed to some undesired variability in daily flow rates. Measurement of instantaneous flow was a poor indicator of daily flow rate, so measurement of composite flow volume was made daily to determine the daily flow rate. Slight adjustments to the speed of the pumps were made when flow volume measurements were not consistent with the target daily flow rate. The tubing was inspected and changed periodically to avoid complete wear through of the tubing from the pump rollers, which would result in slowed flow rates.

### **3.6 Synthetic tile water preparation and monitoring**

A single influent container holding up to 130 liters of solution provided synthetic tile water to each column. Markings were made on the outside of the plastic tub at 10-liter intervals to allow measurement of volume of deionized water transferred into the containers.

Nutrients were measured and dissolved in DI water. Synthetic tile water with target NO<sub>3</sub>-N concentrations of 10 mg/L, 30 mg/L, and 50 mg/L were made.

A recipe of additional micronutrients, based on a nutrient-extraction solution of: 4.0 mM CaCl, 2.0 mM KH<sub>2</sub>PO<sub>4</sub>, 1.0 mM K<sub>2</sub>SO<sub>4</sub>, 1.0 mM MgSO<sub>4</sub>, 25 µM H<sub>3</sub>BO<sub>3</sub>, 2.0 µM MnSO<sub>4</sub>, 2.0 µM ZnSO<sub>4</sub>, 0.5 µM CuSO<sub>4</sub>, 0.5 µM Na<sub>2</sub>MoO<sub>4</sub> (Nadelhoffer, 1990), were measured and added to the solution to reduce the potential effect of micronutrient deficiencies on the denitrifier microbial communities. The synthetic tile water was pumped directly from the influent container to each column. Electrical conductivity of the prepared synthetic tile drainage was monitored and maintained within a range consistent with values observed under average field conditions. Electrical conductivity was measured using a Fisher Scientific accumet® AB30 conductivity meter. KCl was added to the influent solution to maintain an electrical conductivity within a range observed in tile drainage, 600 to 800 µS, while also maintaining where possible nutrient ratios consistent with the nutrient solution. A concentrated stock solution of KCl was prepared at 120 g KCl L<sup>-1</sup> deionized water, and added to the prepared tile drainage solution when electrical conductivity was low (below 600 µS). The quantity of KCl needed to adjust the electrical conductivity was determined experimentally, measuring the change in conductivity of DI water with an incremental addition of KCl to 100 mL DI water. Excel© was used to determine a linear relationship between the concentration of KCl in solution and the electrical conductivity. The resulting equation was:  $y = 1780.9x + 12.25$ , where y is the concentration of KCl (g L<sup>-1</sup>) and x is the resulting electrical conductivity (µS). KCl was not added to the influent solution at a 50 mg L<sup>-1</sup> NO<sub>3</sub>-N concentration, since the initial conductivity measurement for the high nitrate concentration solution exceeded the target electrical conductivity range.

The influent solution pH was also monitored and maintained within a range expected within soil and tile drainage (6.0-7.0). The pH was measured with an Orion model 290A pH meter, and pH was adjusted as needed by adding concentrated NaOH dissolved in DI water to the influent solution. For each 10 L of prepared solution, approximately 1 mL of concentrated NaOH solution was added to the influent solution. Five crystals of NaOH were dissolved in 40 mL DI water to prepare the concentrated NaOH solution. Influent and effluent samples were monitored for characteristics related to denitrification conditions. Conductivity and pH of influent were maintained within levels observed in tile drainage based on lab and field experience (generally near a pH of 6-6.6, and an electrical conductivity between 600–800  $\mu\text{S}$ ). Influent and effluent measurements were taken periodically to monitor potential changes in pH and conductivity due to biological activity within the columns.

Dissolved oxygen (DO) and oxygen reducing potential (ORP) were measured during the controlled temperature phase of the study. DO measurements were taken for 2-3 sample periods at the end of changing conditions (nitrate concentration or temperature). The instantaneous samples were measured for dissolved oxygen using a Fisher Scientific accumet® AP74 handheld meter. The sample flasks were allowed to fill for 45-60 minutes, until the sample depth would cover the probe sensors. The probe was then carefully inserted into the flask, careful not to disturb the effluent tubing or agitate the sample. The probe sensor was carefully positioned near the end of the effluent tubing in an effort to measure DO as closely to in-column concentrations as possible. DO measurements were taken directly from the influent container by submerging the probe into the influent tank for comparison purposes. The mouth of the collection flasks were too small to allow measurements of both

DO and ORP to be taken simultaneously. The ORP measurements were less affected by the timing of the measurement than DO, (before or after DO measurements), therefore (ORP) measurements were taken after DO measurements. ORP was measured using WTW pH 3300i handheld meter with an Electrode SenTix ORP probe. After carefully removing the DO probe from a sample, the ORP probe was inserted similar to the DO probe. ORP measurements were also taken directly from the influent container by submerging the ORP probe into the influent solution for comparison purposes.

### **3.7 Room temperature conditions**

The room temperature phase of the experiments were conducted at temperatures ranging from 21-25.5°C, comparable to tile-drainage temperatures expected in warmer climates and during warm weather rain events when drainage temperatures would be expected to be higher. Temperatures in tile-drained regions throughout the Midwest can reach values observed during the room temperature phase of the study. Observed water temperatures in a field scale bioreactor in northern Iowa during growing season ranged between 8.9°C and 17.1°C, although temperatures occasionally were observed above 20°C. Although not the norm, temperatures observed during a pilot denitrification bioreactor study in central Iowa at the Iowa State University research farm reached did reach values of 20°C or higher during multiple sampling events (Christiansen et al., 2012).

### **3.8 Temperature controlled chamber**

The columns were set-up in a Conviron™ controlled environment chamber located in the U.S. Department of Agriculture's National Lab for Agriculture and the Environment (Ames, Iowa) during the second phase of the study. Temperature was maintained at 10°C,

15°C, and then 20° C to simulate soil temperatures expected at tile drainage depths during a growing season. The influent reservoir, columns, and pumps were contained within the chamber, while the effluent tubing was routed through a port in the chamber door and emptied into the effluent containers. Influent solution was mixed using cooled DI water to reach temperatures within 4 degrees of the ambient temperature within the chamber. 20-liter carboys were filled with DI water and stored in a cooler at 4 degrees Celsius, and mixed with DI water directly from the laboratory source to reach a temperature within 4 degrees of the controlled temperature.

### **3.9 Sample Collection**

Each column drained into individual effluent containers to allow for mass measurement of the effluent flow volume. The effluent containers were weighed at the time of sampling, and weight measurements were converted into volume measurements, assuming 1-liter of effluent solution was equal to 1-kg of mass, to calculate composite flow volumes. The composite effluent containers were measured for total flow volume during both the room temperature and the controlled temperature phases of the study. A 125 ml sample was collected from the composite effluent containers, and used for analyses of NO<sub>3</sub>-N and total organic carbon (TOC) during the room temperature phase of the study. Since the effluent containers had to be housed outside of the controlled environment chamber, instantaneous samples were used for all analyses during the temperature controlled phase of the study. Instantaneous samples were collected daily in flasks outside of the chamber.

The instantaneous samples were collected from the effluent columns tubing directly into 500 ml flasks, and transferred into 125 ml plastic sample bottles after pH, electrical

conductivity, DO, or ORP measurements were made. The samples were then acidified to inhibit any potential microbial activity, and stored at 4°C until further analysis.

### **3.10 Sample Analysis**

Samples were analyzed at the Iowa State University Agriculture and Biosystems Water Quality Research Lab. Collected samples were analyzed with a Cd-reduction method for NO<sub>3</sub>-N + NO<sub>2</sub>-N (Lachat Quick –Chem 800 automated analyzer). Total organic carbon was analyzed using the Hach spectrophotometer and Hach TOC test-n-tube kits for low and medium range samples.

### **3.11 Bromide tracer test**

A tracer test, using a plug of 100 mg L<sup>-1</sup> concentrated KBr<sup>-</sup> solution, was conducted during the first phase of the study to confirm the assumed hydraulic characteristics of the denitrification woodchip bioreactor columns. The tests were run at a theoretical 2-hour retention time, for a total of 8 hours. Effluent samples were taken every 10 minutes for the first 90 minutes, every 5 minutes for the next 110 minutes, then 10 minute intervals for 170 minutes, and at 20 minute intervals for the remainder of the test. Samples were analyzed at Iowa State University's ABE Water Quality Research Lab with Lachat QuickChem 8000 autoanalyzer

### **3.12 Statistical Analysis**

Statistical analysis of the data was conducted using SAS statistical software. Analysis of variance (ANOVA) was conducting using PROC GLM and PROC REG programming. P < 0.05 was used as a significance level. Comparisons were made of differences in nitrate removal between treatments specific to the phase of the study. Analysis of the room-

temperature phase data was conducted between removal at the various HRTs with a relatively consistent influent concentration. Pairwise comparisons were made between removals at each HRT with influent concentrations that were at mean concentration or higher and the concentrations below the mean value for that HRT to discount the effect of the fluctuations in influent concentrations. Likewise, pairwise comparisons were made for data from the temperature controlled study.



## Chapter 4 : Results and Discussion

### 4.1 Column and woodchips characteristics

The columns were designed for use as triplicate column denitrifying woodchip bioreactors, each with identical physical characteristics. However, some variability was observed in the column dimensions, specifically the total volume of each column. These differences in column dimensions resulted in differences in the total woodchip volume, and therefore mass of woodchips per column, and the resulting pore volume in each column. Column 1 had the smallest volume, at 7.61 L, while the volumes of columns 2 and 3 were larger, at 7.74 L and 7.67 L respectively. Column 1 had both the lowest column volume and lowest woodchip mass, but the highest pore volume. This indicated that column 1 was not packed as tightly as columns 2 and 3. This is further supported by the calculated bulk density of the woodchips in each column. Woodchip bulk density was calculated during the first phase of the study using the total volume of the individual columns and the weighed mass of the woodchips. The mean bulk density for the three columns was  $0.343 \text{ g cm}^{-3}$  as moist woodchips. The bulk density of the dry woodchips was  $0.194 \text{ g cm}^{-3}$ .

Visual observation of the packed columns at the start of the temperature controlled phase of the study suggested the columns were packed more tightly than during the room temperature phase, assuming similar woodchip characteristics, however, a mass measurement of the woodchip packed columns indicated a smaller bulk density. The woodchips used for the temperature controlled phase were purchased in early spring, and had likely experienced weathering while stored outdoors at Golden Valley over the winter.

The moisture content of the woodchips ranged from 30.2 to 56.4% with a mean moisture content of 43.2% for the room temperature phase. New woodchips were purchased

from Golden Valley Hardscapes for the temperature controlled phase of the study. Only one pan of woodchips were dried at the start of the temperature controlled phase under the assumption that woodchip moisture characteristics would be similar during both phases of the study, however, the moisture content of the sample dried for the temperature controlled measured considerably lower, at 25.5%, further suggesting the new batch of woodchips may have experienced weathering before purchase. A previous denitrification woodchip bioreactor study, using woodchips from the same supplier, reported woodchip moisture contents of 42.5 to 45.5%, consistent with measurements during the room temperature phase of the study (Christianson et al., 2010). The measured moisture content of the woodchip samples (Table 4.1) was used to estimate the oven-dry weight of the woodchips packed in the columns at the start of the room temperature and temperature controlled phases of the study.

**Table 4.1 Woodchip moisture content determined by measurement of additional WCs**

| Woodchip moisture characteristics |                      |
|-----------------------------------|----------------------|
| Phase 1 - room temperature        | Moisture content (%) |
| sample 1                          | 30.2                 |
| sample 2                          | 42.9                 |
| sample 3                          | 56.4                 |
| mean $\pm$ stdv                   | 43.2 $\pm$ 13.1      |
| Phase 2 - controlled temperature  | 25.5                 |

Due to the differences in column characteristics, the synthetic tile water likely had less physical contact with woodchip surfaces in column 1, and therefore denitrifying bacteria, than the solution passing through columns 2 and 3 at the same HRTs. Column 2 had both the highest column volume and the highest woodchip mass. The resulting pore volume of column 2 was 4.59 L, a mid-range between column 1 and 3 which had pore volumes of and 4.70 and 4.26 L respectively. Column 3 had both the mid-range volume and mid-range

woodchip mass, but the lowest pore volume. The woodchips in columns 2 and 3 were packed to similar woodchip bulk densities.

Under similar conditions, nitrate removal would be expected to be lower in column 1 compared to column 2 and 3 in this study. Reduced denitrification in column 1 was observed throughout the room temperature phase of this study. Columns 2 and 3 would be expected to behave similarly under similar conditions based on the column volume, woodchip mass, and resulting woodchip bulk density in each column. However, a tracer test of the columns suggests column 2 had different hydraulic characteristics than both columns 1 and 3. An earlier breakthrough of the bromide tracer in column 2 suggests short circuiting within the column. There was also a smaller portion of the bromide recovered from column 2. The implication on nitrate removal would be lower overall removal due to reduced contact of the denitrifying bacteria with solution that bypassed the expected flow pattern in the column. Although column 2 generally had higher nitrate removal than column 1, as expected due to the general column and woodchip characteristics, column 2 generally removed less  $\text{NO}_3\text{-N}$  than column 3 under similar conditions (influent concentration and HRT).

The total porosities of individual packed columns (Table 4.2) were calculated as the ratio of measured fluid volume within the woodchip packed column to the total measured volume of the columns. The total porosity calculated ranged from 0.88 to 0.91, and was comparable to a 0.84 reported porosity for fresh hardwood woodchips used in a column denitrification bioreactor study by Robertson et al. (2010).

**Table 4.2 Denitrification woodchip bioreactor column characteristics.**

| Column          | Total Volume(L)  | Pore Volume(L)  | Gravitational Porosity | Secondary Porosity | Total Porosity  |
|-----------------|------------------|-----------------|------------------------|--------------------|-----------------|
| 1               | 7.61             | 4.70            | 0.62                   | 0.29               | 0.91            |
| 2               | 7.74             | 4.59            | 0.59                   | n/a                | 0.89*           |
| 3               | 7.668            | 4.26            | 0.56                   | 0.32               | 0.88            |
| mean $\pm$ stdv | 7.67 $\pm$ 0.065 | 4.52 $\pm$ 0.23 | 0.59 $\pm$ 0.03        | 0.30 $\pm$ 0.02    | 0.89 $\pm$ 0.02 |

\* The average secondary porosity of columns 1 and 3 were used to approximate the secondary porosity of column 2, and therefore calculate the total porosity.

## 4.2 Bioreactor Flow Characteristics

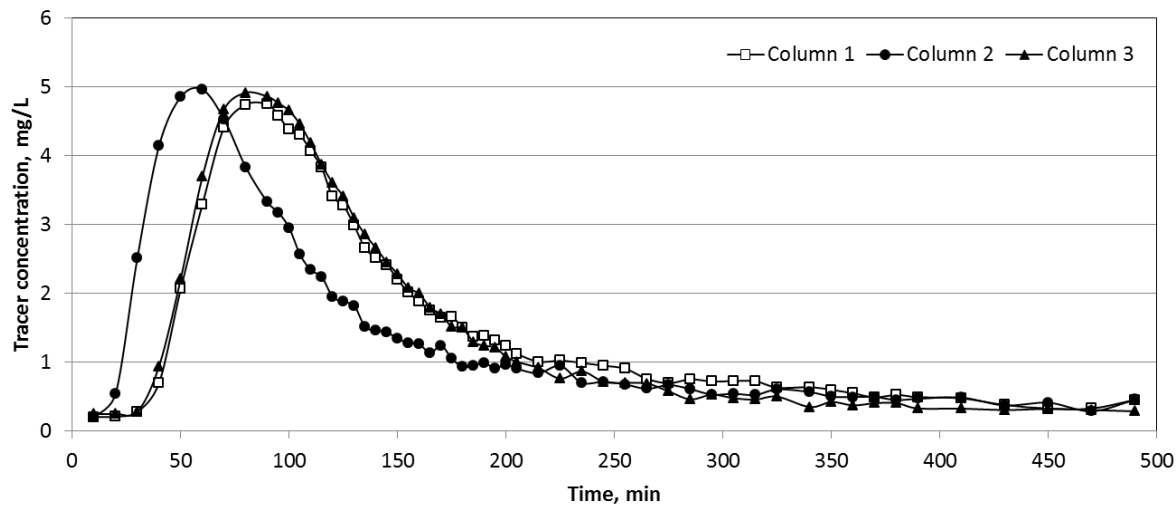
A bromide tracer test was conducted on the three columns to determine bioreactor flow characteristics, such as mean residence time and dispersion index. Figure 4.1 shows the effluent tracer concentration vs. time data for the three columns. Columns 1 and 3 showed similar hydraulic response on the graph, but effluent tracer concentrations in column 1 were higher than column 3 beyond 175 minutes. This resulted in increased mean residence time. Mean residence time for the three columns were between 2.2 and 2.5 hours and showed an increased HRT compared with the theoretical residence times determined by flow rate and pore volumes. The theoretical HRT was estimated based on the collected flow volume during a designated length of time, assuming equal residence time for each particle. In a perfect plug-flow reactor, theoretical HRT and mean residence time would be equivalent. The bromide tracer analysis results indicated the column reactors did not operate as ideal plug-flow reactors, with potential diffusion and short-circuiting within the columns, resulting in a range of residence times for individual particles. A higher mean residence time as determined with tracer analysis in comparison to theoretical values was consistent with tracer test results from previous pilot and field-scale denitrification woodchip bioreactor studies (Christianson et al,2011).

The Morrill Dispersion Index (MDI) was used to evaluate the plug flow characteristics of the column reactors. MDI is defined as:

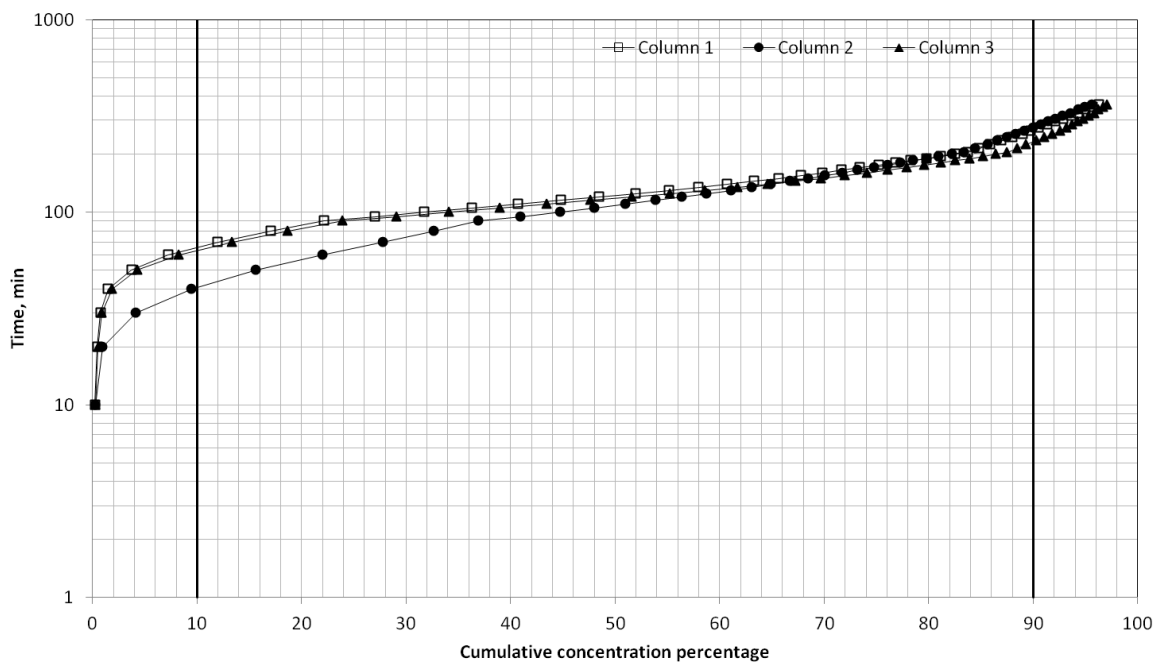
$$\text{MDI} = \frac{P_{90}}{P_{10}} \quad \text{equation 4.1}$$

Where  $P_{90}$  is the time at which 90 percent of the cumulative tracer mass that had eluted the column, and  $P_{10}$  is the time at which 10 percent of the cumulative tracer mass had eluted.

While the MDI value for an ideal plug flow reactor is 1.0, and that for a completely mixed reactor is 22, the US EPA considers an MDI value of 2 or less to represent effective plug flow characteristics (Metcalf and Eddy, 2003). The MDI values shown in Table 4.3 indicate that the column denitrification woodchip bioreactors were operating close to plug flow. The higher MDI value for column 2 indicates a slightly increased dispersion of the tracer. Christiansen et al. (2012) reported low dispersion levels in field scale bioreactors, with MDI values of 3.5 and 4.2, which are comparable to the MDI values for the column bioreactors in this study.



**Figure 4.1** Concentration vs. time tracer response for upflow column denitrification woodchip bioreactors.



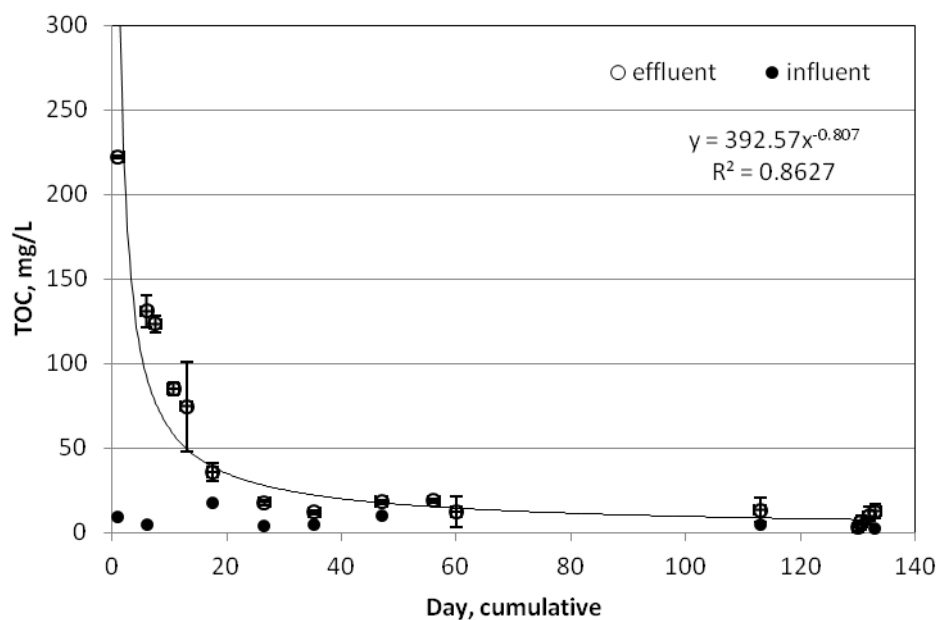
**Figure 4.2**  $P_{10}$  and  $P_{90}$  for bioreactor columns.  $P_{10}$  represents the point where 10 percent of the tracer mass has eluted the columns, and  $P_{90}$  is the point where 90 percent of the tracer mass has eluted.

**Table 4.3 Bioreactor flow characteristics as determined from tracer test analysis.**

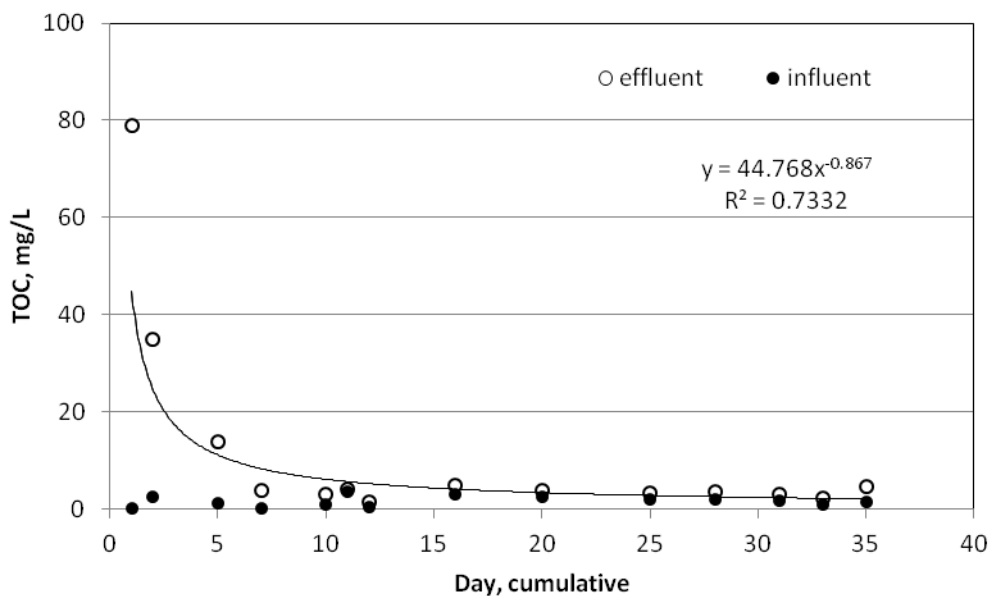
| Column number | Mean residence time | Standard Deviation | MDI = $P_{90}/P_{10}$ | Theoretical residence time |
|---------------|---------------------|--------------------|-----------------------|----------------------------|
| 1             | 150 min.<br>2.5 hr  | 85 min             | 4.15                  | 2.02 hr                    |
| 2             | 138 min.<br>2.3 hr  | 96 min             | 7.00                  | 1.75 hr                    |
| 3             | 134 min.<br>2.2 hr  | 90 min             | 3.54                  | 1.69 hr                    |

### **4.3 Organic Carbon Release and Woodchip Consumption**

There is concern with inserting a relatively large carbon source into the landscape. The release of additional carbon into surface waters that may otherwise be limited by carbon availability, may enhance unwanted microbial growth, which contributes to the depletion of dissolved oxygen. Methylation of heavy metals, specifically mercury, and the production of trihalomethanes are additional concerns with increased organic carbon availability (Malcolm et al., 2010). Total organic carbon (TOC) analysis over the first 4 months of denitrification woodchip bioreactor column operation suggest an initial flush of carbon residues, with a stabilization in carbon release within 50 pore volumes, or approximately 25 days, at a 12-hour HRT (Figure 4.3).



**Figure 4.3 TOC released at start-up of Phase 1 of triplicate denitrification woodchip bioreactor study.** Non-weathered woodchips used to pack the columns for Phase 1 of the study had a high initial flush of TOC. The effluent concentrations dropped to stream background levels within 24 days at a 12-hour HRT.



**Figure 4.4 TOC released at start-up of Phase 2 of the triplicate denitrification woodchip bioreactor study.** Weathered woodchips used to repack the columns for Phase 2 released less TOC. The effluent concentrations dropped to stream background levels within 5 days at a 12-hour HRT.



Another concern with OC inputs is the potential for the formation of disinfection byproducts like trihalomethanes during water treatment. As illustrated in Figure 4.2, the peak concentration of 222 mg/L total organic carbon (TOC) was measured immediately after start-up. TOC concentrations fell to background levels observed in surface streams within approximately 50 pore volumes, or 25 days at a HRT of 12-hours. Background levels in Iowa streams are typically 3-20 mg/L with spikes of 100-600 mg/L. The trendline in Figure 4.2 represents a power function fit to the data, with an  $R^2$  value of 0.88. Based on the TOC release data, it was estimated that approximately 17.0 grams of TOC/kg of woodchip (or 1.70% of woodchip mass) would be released after 1000 pore volumes of flow, i.e. 500 days at an estimated 12-hour HRT. TOC analysis was not conducted for subsequent restarts of the bioreactors; however, the columns did show a brief 1-3 sample periods of initial increase in nitrate removal upon restart, indicating possible increased release of TOC at restarts.

The measured mass loss of the woodchips suggests analysis of TOC release may underestimate the loss and subsequent release of woodchips and carbon from the denitrification woodchip bioreactor columns (Table 4.4). The utilization of carbon as an energy source by the denitrifying bacteria and other microbes within the bioreactor may account for the variability in woodchip loss between the TOC analysis and the woodchip mass measurements and calculations. Assuming the difference in TOC release and mass WC loss can be attributed to microbial consumption, carbon utilized within the bioreactor was approximately 82 grams over 11 months, or  $0.3 \text{ g WC d}^{-1}$ .

The woodchip mass loss from the denitrification woodchip column bioreactors was estimated over approximately 9 months of operation during the room temperature phase of

the study, and 1 month during phase 2. There is a measurable woodchip mass loss of 5.96 to 8.05%, correlating to average monthly woodchip loss of 0.66 to 0.89% over the course of Phase 1 of the study. Measurements of woodchip loss during the 1 month temperature controlled phase are inconclusive, demonstrating a loss of 0.52% and 0.06% in columns 1 and 2, respectively. Measurements of column 3 suggest woodchip mass increased. The discrepancies in values are likely a result of measurement error.

**Table 4.4 Estimated woodchip loss from denitrification woodchip bioreactor columns.**

|                         | Phase 1-room temperature |          |          | Phase 2-temperature controlled |          |          |
|-------------------------|--------------------------|----------|----------|--------------------------------|----------|----------|
|                         | Column 1                 | Column 2 | Column 3 | Column 1                       | Column 2 | Column 3 |
| oven dry WC (start), kg | 1.47                     | 1.51     | 1.49     | 1.49                           | 1.51     | 1.45     |
| oven dry WC (end), kg   | 1.36                     | 1.42     | 1.37     | 1.48                           | 1.51     | 1.46     |
| WC mass loss, kg        | 0.11                     | 0.09     | 0.12     | 0.01                           | 0.00     | -0.01    |
| WC % loss               | 7.48%                    | 5.96%    | 8.05%    | 0.52%                          | 0.06%    | -0.60%   |

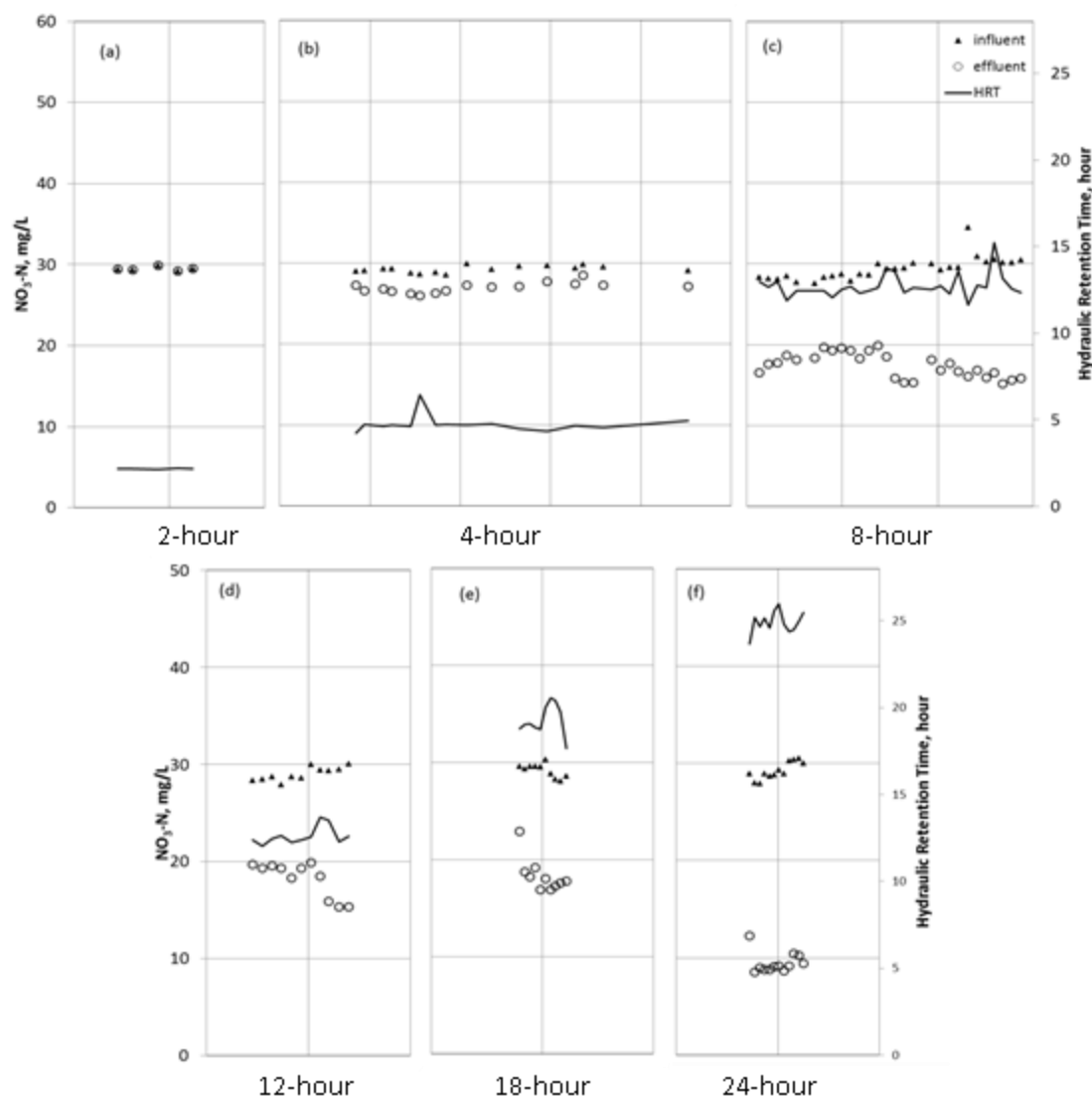
To calculate the moisture content of the woodchips, and estimate the oven-dry woodchip mass of the packed columns, a representative sample of woodchips were oven-dried and weighed at atmospheric equilibrium (cooled to room temperature) at the start of the study. The measured oven-dry mass of the woodchips from each column at the end of the room temperature phase was subtracted from the estimated dry weight of the woodchips at the start of the study to determine the woodchip mass loss. The columns were used in the room temperature phase of the study for a total of 9 months, with varying flow conditions, including completely saturated with no flow, drained with no flow, and the periods with HRTs of 2, 4, 8, 12, 18, and 24-hours. During this time, the mean percentage of woodchip loss was 7.17%, 5.47% greater than the calculated TOC release. The woodchip mass loss

estimated by woodchip measurements is consistent with a mass loss of 8.4% at a depth of saturation, conditions most similar to the saturated conditions of the denitrification woodchip bioreactor columns, over the course of a 1 year field-scale bioreactor study. Woodchip mass loss in field scale denitrification bioreactors at depth of saturation have been documented at 8.4 to 13% (Moorman et al., 2010)

The majority of organisms capable of breaking down cellulose and lignin, the primary components of woodchips, into microbial available carbon require aerobic conditions (Sylvia et al., 2005). Therefore, decomposition of organic matter, such as woodchips, is inhibited by saturated conditions (Moorman et al., 2010). Higher percentages of woodchip mass loss would be expected in unsaturated woodchip bioreactors.

#### **4.4 Effect of HRT on Nitrate Removal**

Various extents of nitrate removal were observed depending on the HRT at which the columns were operated. Figure 4.4 shows nitrate removal at target HRTs of 2, 4, 8, 12, 18, and 24-hours in the denitrification woodchip bioreactors as represented by influent and effluent nitrate concentrations. As illustrated in figure 4.4(a), influent and effluent nitrate concentrations were similar, with overlapping data points at an HRT of 2-hours, indicating little nitrate removal. At a 2-hour HRT, the synthetic tile drainage solution was in contact with the woodchip surfaces, and therefore the denitrifying bacteria, for a short period of time. While denitrification activity may have been actively occurring within the column bioreactors, the reduced physical contact of the solution may have limited the percent of nitrate removal possible.

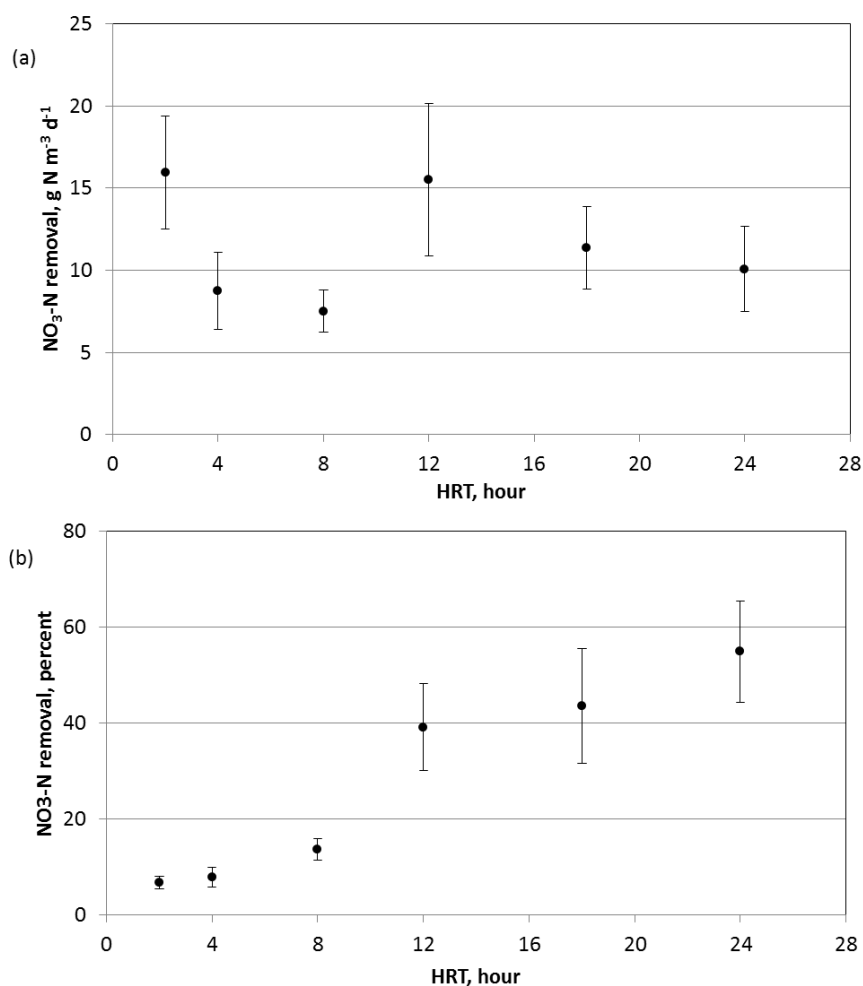


**Figure 4.5 Nitrate removal at HRTs from 2 to 24-hours. The solid line indicates HRT values calculated from measured flow rate and pore volume. The space between each horizontal line represents 20 pore volumes at the specified HRT.**

Nitrate percent removal was observed to increase with increased hydraulic retention time. At a target HRT of 4-hours, influent concentration between 28.6 and 30.0 mg L<sup>-1</sup> resulted in effluent nitrate concentrations of 24.9 to 29.6 mg L<sup>-1</sup>, demonstrating a 7.8% mean removal with a standard deviation of 2.1. Although field scale bioreactors are designed for

higher retention times, during large flow events achieved HRT may be at 4 hours or less. This study suggests that removal rates during high flow periods may be as low as  $6.6 \% \pm 1.3$ . It is anticipated that such flows occur only a few times during the year.

As HRT was increased to 8-hours (Fig.4.4(c)), influent nitrate concentrations of 29.0 to 32.1 mg L<sup>-1</sup> were reduced to effluent nitrate concentrations of 23.9 to 28.3 mg L<sup>-1</sup>. The resulting percent removals are shown in Figure 4.5(b). At the 8-hour HRT, percent removal was 13.6 % with a standard deviation of 2.3. Further increasing the HRT to 12-hours (Fig. 4.4(d)) resulted in increasingly lower effluent nitrate concentrations. The influent nitrate concentrations of 27.6 to 34.5 mg L<sup>-1</sup> resulted in effluent nitrate concentrations of 9.9 to 23.2 mg L<sup>-1</sup>. The mean percent removal at 12-hour HRT was 46.2 %, with a standard deviation of 7.8. Nitrate removal at 18-hour HRT and 24-hour HRT were not significantly different from the 12-hour HRT, based on ANOVA comparisons for all target HRTs (SAS, 2009). The influent concentrations at 18-hour and 24-hour were within the range 28.1 to 30.3 mg L<sup>-1</sup>, and 27.9 to 30.5 mg L<sup>-1</sup>, respectively. The corresponding effluent concentrations were 9.9 to 20.8 mg L<sup>-1</sup> and 8.5 to 24.5 mg L<sup>-1</sup>, respectively. The resulting percent removal at 18-hour HRT was 43.5 %, with a standard deviation of 12.0. At 24-hour HRT, the removal was 54.9 %, with a standard deviation of 10.6.



**Figure 4.6 Nitrate (a) load removal, and (b) percent removal at 2 to 24-hour HRTs with similar influent concentrations.**

Nitrate load removal at various HRTs with a NO<sub>3</sub>-N influent concentration of approximately 30 mg L<sup>-1</sup> at room temperature. Nitrate load removal in g N m<sup>-3</sup> d<sup>-1</sup> is illustrated in Figure 4.4(a) and nitrate percent removal (b). To reduce the influence of flow volume within a given HRT on nitrate load, the load values were normalized to expected flow volume at each target HRT for statistical analysis. An ANOVA comparison was conducted of the differences in removal between each HRT. The product of expected flow volume and nitrate removal, in mg L<sup>-1</sup>, was used to calculate the expected load removal in g

$\text{N d}^{-1}$  (Figure 4.5). The highest nitrate load removal was observed at 2 and 12-hour HRTs, with statistically identical at 2-hour and 12-hour HRTs. There was no significant difference in load removal between the target 4, 8, and 24-hour HRTs. Additionally, there is no significant difference in load removal between 18 and 24-hour HRTs.

The target HRTs and subsequent  $\text{NO}_3\text{-N}$  load removals are depicted in Table 4.5. While the achieved HRTs were somewhat lower than the desired target HRTs, the increment of time between each increase in HRT were relatively consistent with the desired HRT change. The target HRTs were selected to demonstrate  $\text{NO}_3\text{-N}$  removal as HRT doubled from 2 to 4-hour HRT, 4 to 8-hour HRT, and 12 to 24-hour HRTs. The 18-hour target HRT was selected to demonstrate  $\text{NO}_3\text{-N}$  removal at a mid-range between the 12 and 24-hour HRTs.

The total volume of solution treated at a 4-hour HRT would be half the volume treated at a 2-hour HRT. Subsequently, the load removal would be expected to also halve if the removal rates were similar.  $\text{NO}_3\text{-N}$  load removal was near 50% in columns 1 and 3, with 48% and 46% respectively. Removal in column 2 was approximately 9% lower than would be expected based on the reduced flow volume, with only a 41% reduction in  $\text{NO}_3\text{-N}$  load. The similar reductions in flow volume treated and  $\text{NO}_3\text{-N}$  load removal suggest no marked improvement in removal efficiency from 2-hour to 4-hour HRT.

The flow volume was further reduced by half from the 4-hour to 8-hour HRT; however, the load removal did not decrease proportionally, indicating improved  $\text{NO}_3\text{-N}$  removal efficiency from the 4-hour to 8-hour HRT. It was assumed that DO concentrations were initially too high to support denitrification in the woodchip bioreactor columns at the

low HRTs, as the more swiftly flowing solution carried increased amounts of DO. In addition, denitrifier biomass accumulation may have been hampered as biomass was also transported at the higher flow rates. As HRT was increased, less DO was transported into the columns while more DO was removed within the columns via biological activity. With  $O_2$  no longer available, the denitrifiers utilized the available  $NO_3-N$ . The conditions within the column bioreactor appeared to support exponential growth of denitrifying bacteria, with the load removal increasing from the 8 to 12-hour HRT. Although the flow volume decreased from the 8-hour to 12-hour HRT, there was an increase in load removal.

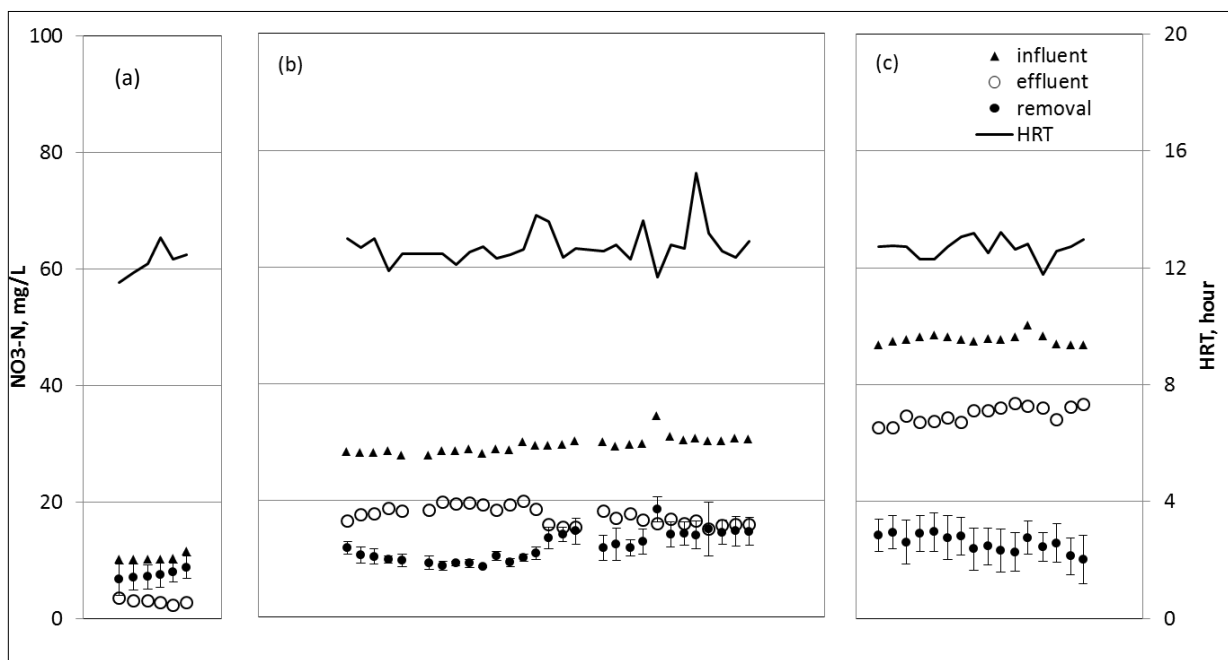
**Table 4.5 Target HRTs and  $NO_3-N$  load removal normalized to expected flow volume at room temperature.**

| HRT | $NO_3-N$ removal (normalized to target HRT) |                  |                  |
|-----|---|------------------|------------------|
| (h) | $(g\ N\ m^{-3}\ d^{-1})$                    |                  |                  |
|     | Column 1                                    | Column 2         | Column 3         |
| 2   | $14.48 \pm 2.23$                            | $13.63 \pm 1.90$ | $19.75 \pm 2.22$ |
| 4   | $7.56 \pm 1.96$                             | $8.01 \pm 1.56$  | $10.76 \pm 2.10$ |
| 8   | $7.25 \pm 0.98$                             | $6.81 \pm 1.03$  | $8.46 \pm 1.31$  |
| 12  | $12.82 \pm 2.18$                            | $14.71 \pm 3.30$ | $18.14 \pm 5.16$ |
| 18  | $10.07 \pm 0.82$                            | $9.41 \pm 1.04$  | $14.67 \pm 0.62$ |
| 24  | $8.02 \pm 1.62$                             | $8.9 \pm 0.94$   | $13.22 \pm 0.63$ |

#### 4.5 Effect of influent nitrate concentration on nitrate removal

The effect of influent concentration on  $NO_3-N$  load removal and concentration reduction was evaluated during both the room temperature and controlled temperature phases of the study. Nitrate removal at the target 12-hour HRT of the room temperature phase is demonstrated in Figures 4.5 and 4.6. Although percent removals were different with varying influent concentrations at a 12-hour HRT, the load reductions ( $mg\ L^{-1}$  and  $g\ N\ m^{-3}d^{-1}$  removed) were less variable (Figures 4.5 and 4.6).





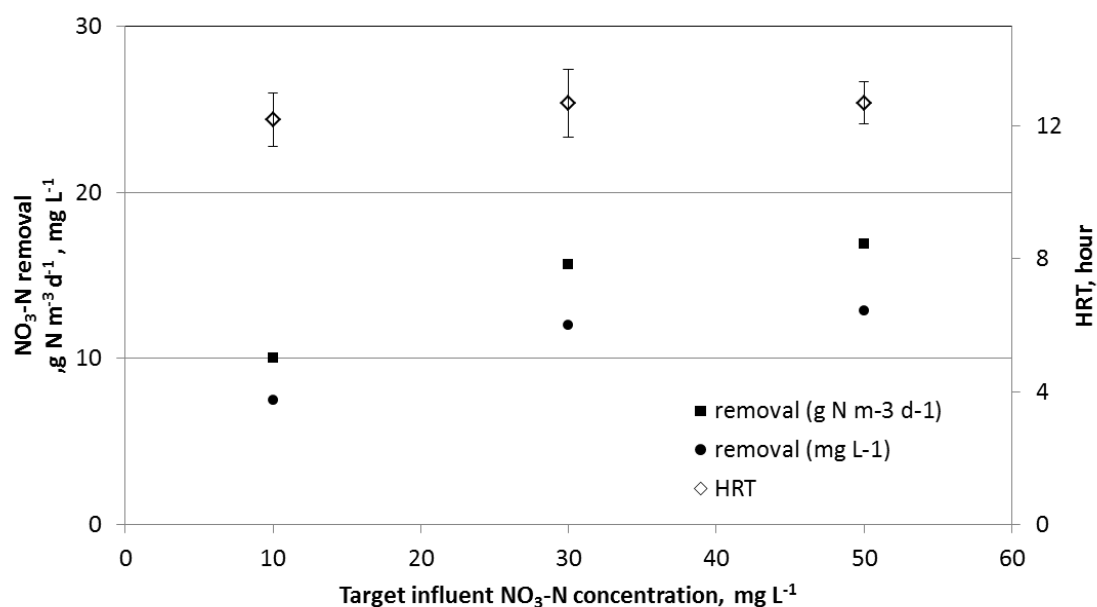
**Figure 4.7 Comparison of nitrate removal at 10, 30, and 50 mg L<sup>-1</sup> influent nitrate concentrations.**

Figure 4.6 illustrates the NO<sub>3</sub>-N load removal at influent concentrations of (a) 10 mg/L, (b) 30 mg/L, (c) and 50 mg/L at a target HRT of 12-hours during the room temperature phase of the experiment. Samples were taken daily, and represent the composite sample of two pore volumes. Solid circles indicate nitrate removal as mg/L, with standard deviation noted.

The load reduction at a 10 mg L<sup>-1</sup> was significantly lower than the load reductions at both 30 mg L<sup>-1</sup> and 50 mg L<sup>-1</sup> target influent concentrations. There was no significant difference in load reductions at the higher influent concentrations. Additional nitrate removal at these higher concentrations seems to be limited by factors other than nitrate

availability. Previous studies suggest denitrifier biomass and carbon availability may limit denitrification when sufficient nitrate is available (Moorman et al, 2010).

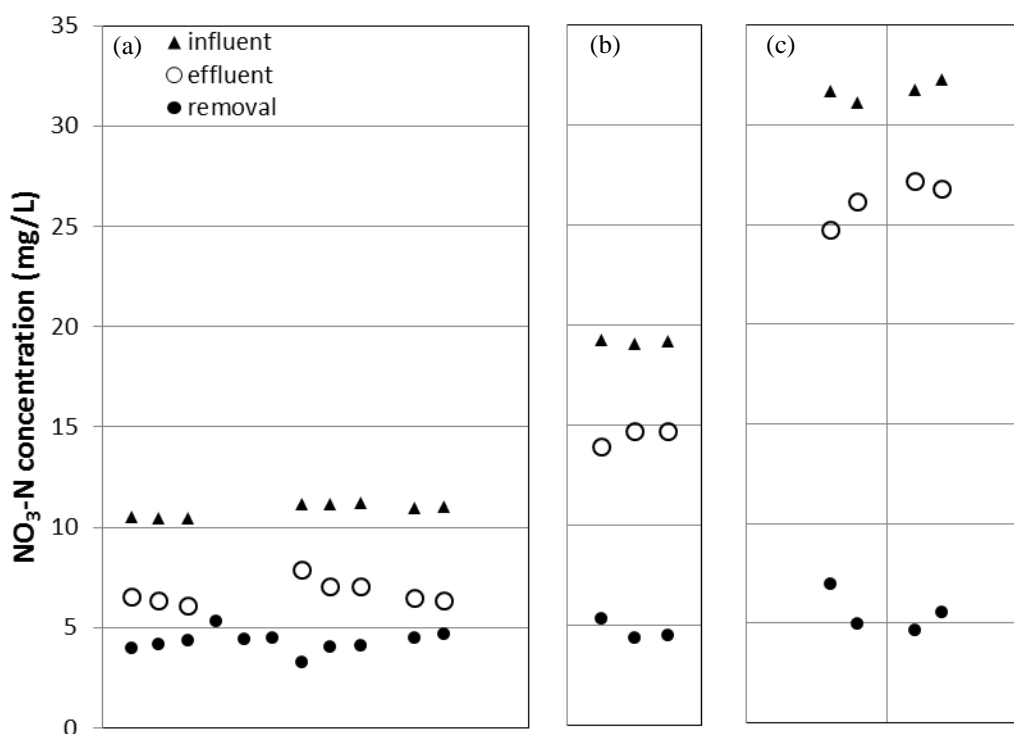
A comparison of mean  $\text{NO}_3\text{-N}$  removal at the 10, 30, and 50  $\text{mg L}^{-1}$  (Figure 4.7) highlights the reduced increase in load removal with increased concentration. During the target 12-hour HRT sampling periods, nitrate removal with influent concentrations of 30  $\text{mg L}^{-1}$  and 50  $\text{mg L}^{-1}$  were similar. A mean removal of 12.0  $\text{mg L}^{-1}$  was observed with influent nitrate concentrations between 27.6  $\text{mg L}^{-1}$  and 34.5  $\text{mg L}^{-1}$  (mean  $29.4 \pm 1.4 \text{ mg L}^{-1}$ ). With influent concentrations between 46.7  $\text{mg L}^{-1}$  and 50.2  $\text{mg L}^{-1}$  (mean  $47.8 \pm 0.9 \text{ mg L}^{-1}$ ), nitrate removal was  $12.9 \pm 3.1 \text{ mg L}^{-1}$ . Comparison of mean nitrate removal for various influent nitrate concentrations show lower nitrate removal with a lower nitrate concentration of 10  $\text{mg L}^{-1}$ . The mean HRT during the sampling period at 10  $\text{mg L}^{-1}$  was 12.2 hours, with a standard deviation of 0.8 hours. During the 30  $\text{mg L}^{-1}$  and 50  $\text{mg L}^{-1}$  sampling periods, mean HRTs of 12.7 hours, with standard errors of 1.0 hours during the 30  $\text{mg L}^{-1}$  target period, and 0.63 hours during the 50  $\text{mg L}^{-1}$  sampling period. Since the HRT was held relatively steady for the comparisons of  $\text{NO}_3\text{-N}$  removal at the various influent concentrations, there was minimal effect of flow volume on differences in  $\text{NO}_3\text{-N}$  mass removal in terms of  $\text{g N m}^{-3}\text{d}^{-1}$ . The removal rate at the target 10  $\text{mg L}^{-1}$  influent concentration was  $10.03 \pm 1.89$  of  $\text{g N m}^{-3}\text{d}^{-1}$ . The target 30 and 50  $\text{mg L}^{-1}$  influent concentration samples showed similar load removals, with  $15.64 \pm 3.53$  and  $16.91 \pm 4.26$  of  $\text{g N m}^{-3}\text{d}^{-1}$ , respectively. Evaluation of nitrate removal at a controlled  $10^\circ\text{C}$  also demonstrates an increase in removal with increased influent  $\text{NO}_3\text{-N}$  concentrations of 10, 20, and 30  $\text{mg L}^{-1}$  (Table 4.6). Figure 4.7 further illustrates the load removal at response to influent concentration at  $10^\circ\text{C}$ .



**Figure 4.8 Comparison of NO<sub>3</sub>-N removal at influent concentrations of 10, 30, and 50 mg L<sup>-1</sup>.**

**Table 4.6 Comparison of NO<sub>3</sub>-N removal with 10, 20, and 30 mg L<sup>-1</sup> influent at 10° C.**

| Removal Response to Influent Concentration |        |      |                                   |      |                       |      |
|--|--------|------|-----------------------------------|------|-----------------------|------|
| Influent Conc.(mg/L)                       | (mg/L) | stdv | (mg NO <sub>3</sub> -N/Kg WC/day) | stdv | (g N/cubic meter/day) | stdv |
| 10   | 4.13   | 0.30 | 23.13                             | 1.93 | 6.13                  | 0.41 |
| 20   | 4.73   | 0.24 | 25.97                             | 1.68 | 6.94                  | 0.34 |
| 30   | 5.61   | 0.52 | 30.60                             | 2.13 | 8.27                  | 0.48 |

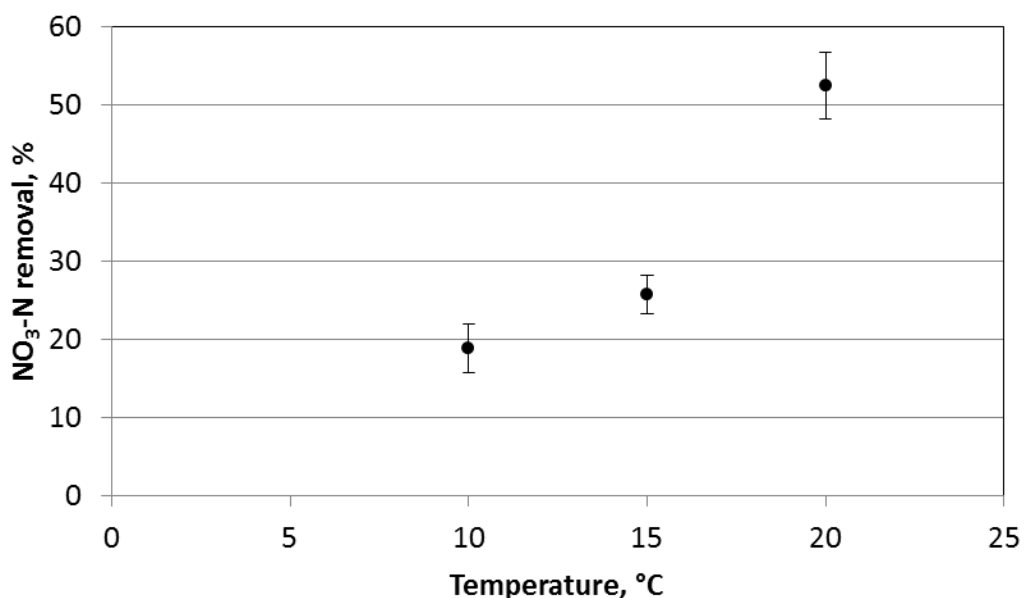


**Figure 4.9 Comparison of NO<sub>3</sub>-N removal for (a) 10, (b) 20, and (c) 30 mg L<sup>-1</sup> influent concentrations at 10° C and a target 12-hour HRT.**

These observations support the hypothesis that under similar environmental and hydraulic conditions, nitrate mass removal is increased when influent concentrations are increased from relatively low concentrations to high concentrations. The increase in removal rates appear to slow with increased influent concentration from 30 to 50 mg L<sup>-1</sup>. No significant increase in nitrate removal was observed between 30 mg L<sup>-1</sup> and 50 mg L<sup>-1</sup> (both high nitrate concentrations relative to tile drainage) at room temperature. The controlled temperature phase of the study did not experience a lag in NO<sub>3</sub>-N load removal from 20 mg L<sup>-1</sup> to 30 mg L<sup>-1</sup>, suggesting nitrate may be the limiting factor to 30 mg L<sup>-1</sup>. Other factors, such as denitrifier biomass or carbon availability may limit denitrification at the higher influent concentrations.

#### 4.6 Effect of temperature on nitrate removal

During the temperature controlled phase of the study, temperature was held to 10°, 15°, and 20°C with a relatively steady achieved HRT of  $12.81 \pm 0.74$  hours. The small variation in HRT throughout the course of the temperature controlled phase of the study did not have a significant impact on nitrate removal within the columns. Nitrate removal showed a stepped increase with temperature (Figure 4.8). Although removal rates are evaluated at only 3 temperatures, making absolute determination of a trend difficult, the results do suggest a potential exponential response in denitrification to increased temperature.



**Figure 4.10 Nitrate percent removal trend with increased temperature at an influent concentration of 30 mg L<sup>-1</sup>.**

Nitrate removal efficiency was evaluated at all temperatures with a target influent concentration of 30 mg L<sup>-1</sup>. The measured influent NO<sub>3</sub>-N concentrations ranged from 27.16 to 32.20 mg L<sup>-1</sup>, with a mean value of  $30.70 \pm 1.58$  mg L<sup>-1</sup>. Resulting effluent concentrations ranged from 20.27 mg L<sup>-1</sup> to 27.62 mg L<sup>-1</sup>, with a mean effluent NO<sub>3</sub>-N concentration of

24.85  $\pm$  2.01 mg L<sup>-1</sup>. Nitrate removal at the controlled 10°C was 18.32 % with a standard deviation of 3.12%. At 15°C, nitrate removal increased significantly to 25.71  $\pm$  2.48%, a total increase of 7.39%. A further increase in denitrification to 52.49%  $\pm$  4.2 was observed at an increased temperature of 20°C. Influent concentrations at 15° and 20° C ranged from 31.76 mg L<sup>-1</sup> to 33.62 mg L<sup>-1</sup> at 15°C and 26.66-30.08 mg L<sup>-1</sup> at 20°C, with mean values of 32.96  $\pm$  0.51 mg L<sup>-1</sup> and 28.33  $\pm$  1.16 mg L<sup>-1</sup>. The resulting mean effluent NO<sub>3</sub>-N concentrations were 24.48  $\pm$  0.09 mg L<sup>-1</sup> at 15°C and 13.45  $\pm$  1.24 mg L<sup>-1</sup> at 20°C. The fluctuation of the influent NO<sub>3</sub>-N concentration around the 30 mg L<sup>-1</sup> target concentration did not significantly impact the differences in nitrate removal observed between temperatures. The NO<sub>3</sub>-N removal percentages show an exponential trend, consistent with trends observed in a sawdust filled column study for nitrate removal in septic systems (Robertson et al.,2010).

Analysis of NO<sub>3</sub>-N removal vs. temperature in a large denitrification bioreactor for treatment of tile drainage suggests an approximate Q<sub>10</sub> factor of 2, with a temperature increase from 5 to 10°C (Elgood, et al., 2010). Results from the current study are reported in Table 4.7, with variable Q<sub>10</sub> factors for comparison of rate of removal at 10°, 15°, and 20°. Both carbon availability and temperature were determined as the limiting factors to denitrification (Warneke et al., 2011). This illustrates that temperature is not the only influential factor in Q<sub>10</sub> determination.

As temperature is increased within a system, factors such as DO were impacted. Solution at warmer temperatures can absorb lower quantities of DO, resulting in lower DO concentrations which support increased denitrification (Veraart et al., 2011). Q<sub>10</sub> factors are therefore influenced by both an increase in general microbial activity expected at higher

temperatures, and the enhanced denitrification observed with lower DO concentrations. The current denitrification woodchip bioreactor column study demonstrated a higher  $Q_{10}$  factor, based on removal rate as determined in  $\text{mg L}^{-1}$  with a temperature increased from  $15^{\circ}\text{C}$  to  $20^{\circ}\text{C}$  ( $Q_{10}=3.08$ ). Results indicate that sufficient carbon was available to support denitrification, and did not inhibit nitrate removal within the columns.  $Q_{10}$  values were calculated for the change in denitrification rates for the three temperatures observed during the temperature controlled phase of the study. The  $Q_{10}$  values calculated based on removal in terms of  $\text{mg L}^{-1}$  do not factor in some variability in total removal due to differences in sampling time. Rates of removal were normalized to a 24-hour period for calculations in terms of  $\text{g N m}^{-3} \text{d}^{-1}$ . The  $\text{mg NO}_3\text{-N kg}^{-1} \text{WC d}^{-1}$  calculations correct for variances in the packed woodchip densities of the columns and normalize for a 24-hour period. These  $Q_{10}$  values are consistent with a  $Q_{10}$  of 2.1 used in a field-scale denitrification woodchip bioreactor study with removal in  $\text{g N m}^{-3} \text{d}^{-1}$  (Warneke et al., 2011). Christianson et al. also reports similar  $Q_{10}$  values for a pilot scale denitrification woodchip bioreactor study (2010).

**Table 4.7  $Q_{10}$  values based on various methods of removal rate calculation.**

| $Q_{10}$ values based on various methods of removal rate calculation |  |                                       |                        |
|--|--|---------------------------------------|------------------------|
| Temperature  | ( $\text{mg NO}_3\text{-N Kg}^{-1} \text{WC d}^{-1}$ ) | ( $\text{g N m}^{-3} \text{d}^{-1}$ ) | ( $\text{mg L}^{-1}$ ) |
| ( $^{\circ}\text{C}$ )   | $Q_{10}$ (unitless)                                    | $Q_{10}$ (unitless)                   | $Q_{10}$ (unitless)    |
| 10 to 15   | 2.30   | 2.23                                  | 2.28                   |
| 15 to 20   | 2.86   | 2.89                                  | 3.08                   |
|  |  |                                       |                        |
| 10 to 20   | 2.56   | 2.54                                  | 2.65                   |

## **Chapter 5 : Conclusions and suggestions for additional research**

### **5.1 Conclusions**

Denitrification woodchip bioreactors are a remediation tool for nitrate removal from agricultural tile drainage, and should be designed to remove the maximum quantity of nitrate feasible. This study has investigated several of the factors that influence denitrification, including HRT,  $\text{NO}_3\text{-N}$  availability, and temperature under controlled laboratory conditions. Variability in nitrate removal efficiency was observed between columns, most notably at room temperature, highlighting the complexity of denitrification. Although exact rates of  $\text{NO}_3\text{-N}$  removal cannot be translated from system to system, general trends can be used to estimate denitrification response to various environmental conditions. Results from this study can provide tools for improving the future design of denitrification woodchip bioreactors for specific climatic conditions and existing nitrate loads. Based on a combination of expected flow volumes, temperature, and nitrate concentration history, land owners and engineers can determine the proportion of drainage to treat, and the optimal hydraulic retention time required. Additionally, this study provides evidence to suggest that using weathered woodchips as a denitrification bioreactor packing material may reduce initial carbon losses to surface waters while maintaining denitrification function.

The potential increase in organic loading of carbon from denitrification bioreactors appears to be minimal. Carbon release was monitored duration the room temperature phase of the study, with results indicating that carbon in effluent within expected background concentration levels after approximately 25 days of operation. Although direct release of carbon does not appear to be an ongoing concern with denitrification woodchip bioreactors,



additional research into the microbial activity within the bioreactor, including incubation of potential pathogens, is needed.

Results from this study support the hypothesis that nitrate load removal increases with increased HRT. The rate of increase, however, declined at HRTs above 12-hours. Above an approximate 18-hour HRT, the increase in rate of removal appears to level off. The study further suggests that denitrification at higher nitrate concentrations is limited by a factor other than  $\text{NO}_3\text{-N}$  availability, such as a set maximum denitrifier population density under specific conditions. In contrast, load reductions in  $\text{g N m}^{-3}\text{d}^{-1}$  removal did not follow a definite trend. The load reductions at 2 and 12-hour HRTs were statistically identical. Although the small reduction of 7% was observed at the 2-hour HRT, the increased volume of synthetic drainage solution treated resulted in a large total load removal. The increased percent removal observed at the 12-hour HRT resulted in a large total removal despite a six-fold reduction in total flow volume treated.

There was a significant decrease in nitrate load removal from 2 to 4-hour HRT. The reduction in load removal can be attributed to the reduced volume of solution treated at the 4-hour HRT, since both the flow volume and nitrate load removal were reduced by 50% from the 2 hour to 4 hour HRT. The flow volume was further reduced by 50% from the 4-hour HRT to the 8-hour HRT; however, the resulting load reduction is not significantly different from the load reduction at the 4-hour HRT. The percent removal is significantly greater at the 8-hour HRT, indicating that the volume of flow is the main factor in the insignificant change in load removal. Nitrate removal appears most efficient at a 12-hour HRT. Both nitrate % removal and load removal are in the highest range for

this study at the 12-hour HRT. Results from the room temperature phase of this study suggest that a bioreactor designed to simultaneously treat the majority of drainage volume at an HRT of 12-hours and the entire flow volumes of high flow storm events at a reduced HRT may provide the best  $\text{NO}_3\text{-N}$  removal results.

$Q_{10}$  values were calculated to provide a means for estimating denitrification at various temperatures observed in the field. This study suggests  $Q_{10}$  factors can be variable within a system dependent on multiple factors, including carbon and nitrate availability, and denitrifier population dynamics. Because of the variability of environmental conditions,  $Q_{10}$  factors can only provide a rough estimation of  $\text{NO}_3\text{-N}$  removal with increased temperature.

## **5.2 Future research**

Denitrifying bacteria research has focused on identifying the mechanisms (enzymes) responsible for bacterial denitrification (Gruntzig et al., 2001; Henry et al., 2004; Michotey et al., 2000; Heylen et al., 2006). Additional research is needed to gain a better understanding of the dynamics of denitrifying bacteria communities. An in-depth understanding of specific growth rates and dynamics of denitrifying bacteria communities could provide insight into the maximum potential denitrification within soils and denitrifying woodchip bioreactors.

There is limited research available documenting denitrification bacteria response to total nitrate load conditions. A focused study analyzing changes in  $\text{NO}_3\text{-N}$  removal rates at high and low influent nitrate loads, as determined by concentration and/or flow

rates, would provide valuable insight into the mechanisms of nitrate removal in denitrification bioreactors.

In addition, research into the impact of low HRT on nitrate removal would be beneficial. Observed nitrate concentrations within tile drainage are often below the 10 mg L<sup>-1</sup> standard set for drinking water, however, aquatic health is impacted by much lower nitrate concentrations (Black et al., 2011). At lower nitrate concentrations, nitrate appears to be the limiting factor. Treatment of tile drainage at a lower HRT during periods of lower nitrate concentration may provide beneficial nitrate removal while avoiding undesirable end-products, such as reduced sulfate, that may occur with the more complete nitrate removal expected at higher HRTs.

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## Appendix A: Raw Data

| Date - time   | PV<br>cum. | target 2-hour HRT   |     |     | [NO3-N]  |          |      |      |
|---------------|------------|---------------------|-----|-----|----------|----------|------|------|
|               |            | HRT based on comp Q |     |     | influent | effluent |      |      |
|               |            | C1                  | C2  | C3  |          | C 1      | C 2  | C 3  |
|               |            | h                   | h   | h   |          | mg/L     | mg/L | mg/L |
| 7/10/10 - 3PM | 286        | 1.8                 | 1.8 | 1.6 | 29.4     | 27.8     | 27.9 | 27.3 |
| 7/10/10 - 9PM | 289        | 1.8                 | 1.8 | 1.6 | 29.4     | 27.8     | 27.7 | 27.2 |
| 7/11/10 - 7AM | 295        | 1.8                 | 1.8 | 1.6 | 29.9     | 27.9     | 28.3 | 27.0 |
| 7/11/10 - 3PM | 300        | 1.9                 | 1.8 | 1.6 | 29.2     | 27.4     | 27.6 | 26.9 |
| 7/11/10 - 9PM | 303        | 1.9                 | 1.8 | 1.6 | 29.5     | 27.2     | 27.4 | 27.1 |

| Date - time        | PV<br>cum. | target 4-hour HRT   |     |         | [NO3-N]  |          |      |      |
|--------------------|------------|---------------------|-----|---------|----------|----------|------|------|
|                    |            | HRT based on comp Q |     |         | influent | effluent |      |      |
|                    |            | C1                  | C2  | C3      |          | C 1      | C 2  | C 3  |
|                    |            | h                   | h   | h       |          | mg/L     | mg/L | mg/L |
| 8/27/10 - 10:30AM  | 360        | 3.6                 | 3.3 | 3.0     | 29.0     | 28.0     | 26.6 | 27.4 |
| 8/27/10 - 6:15PM   | 362        | 3.8                 | 3.8 | 3.4     | 29.1     | 26.7     | 26.3 | 26.9 |
| 8/28/10 - 10:45AM  | 367        | 3.8                 | 3.6 | 3.3     | 29.3     | 27.0     | 26.6 | 26.9 |
| 8/28/10 - 6:10PM   | 369        | 3.7                 | 3.8 | 3.4     | 29.3     | 26.9     | 26.6 | 25.9 |
| 8/29/10 - 10:40AM  | 373        | 3.7                 | 3.7 | 3.4     | 28.8     | 26.6     | 26.4 | 25.7 |
| 8/29/10 - 6:55PM   | 376        | 5.2                 | 5.0 | 4.8     | 28.6     | 26.4     | 26.7 | 24.9 |
| 8/30/10 - 9AM      | 380        | 3.9                 | 3.6 | 3.5     | 28.9     | 26.6     | 26.7 | 25.6 |
| 8/30/10 - 6PM      | 382        | 3.8                 | 3.7 | 3.4     | 28.6     | 26.8     | 26.9 | 26.0 |
| 8/31/10 - 12:30 PM | 387        | 3.8                 | 3.7 | 3.4     | 30.0     | 27.7     | 27.7 | 26.4 |
| 8/31/10 - 6:15 PM  | 389        | 3.9                 | 3.8 | 3.5     | 29.6     | 27.7     | 27.5 | 26.2 |
| 9/1/10-10:35AM     | 393        | 3.8                 | 3.8 | 3.4     | 29.2     | 27.2     | 27.3 | 26.7 |
| 9/1/10-6:45PM      | 395        | 3.9                 | 3.7 | 3.5     | 29.4     | 26.9     | 27.0 | 25.6 |
| 9/2/10-10:45AM     | 400        | 3.4                 | 3.6 | 3.4     | 29.6     | 27.5     | 27.3 | 26.5 |
| 9/2/10-6:40PM      | 402        | 3.7                 | 3.7 | 3.5     | 29.6     | 27.5     | 27.7 | 26.5 |
| 9/3/10-11:20AM     | 407        | 3.5                 | 3.4 | 3.2     | 29.7     | 27.7     | 28.2 | 27.4 |
| 9/3/10-6:45PM      | 409        | 3.9                 | 3.7 | 3.5     | 29.5     | 27.5     | 27.6 | 26.4 |
| 9/4/10-11:40AM     | 413        | 3.8                 | 3.7 | 3.3     | 29.4     | 27.6     | 27.8 | 27.0 |
| 9/4/10-6:40PM      | 415        | 3.8                 | 3.6 | 3.4     | 29.8     | 29.6     | 27.6 | 28.2 |
| 9/5/10-12:15PM     | 420        | 3.9                 | 3.5 | 3.3     | 29.6     | 27.4     | 27.5 | 26.9 |
| 9/5/10-6:05PM      | 422        | 4.0                 | 3.9 | 3.7     | 29.5     | 27.3     | 27.4 | 26.5 |
| 9/6/10             | 428        | 3.8                 | 3.7 | no data | 29.5     | 27.6     | 27.7 | 27.0 |
| 9/7/10             | 434        | 4.0                 | 4.1 | 3.7     | 29.3     | 27.6     | 27.5 | 26.8 |
| 9/8/10             | 441        | 4.0                 | 3.9 | 3.6     | 29.1     | 27.4     | 27.4 | 26.7 |

| Date - time | PV<br>cum. | target 8-hour HRT   |         |      | [NO3-N]  |          |         |      |
|-------------|------------|---------------------|---------|------|----------|----------|---------|------|
|             |            | HRT based on comp Q |         |      | influent | effluent |         |      |
|             |            | C1                  | C2      | C3   |          | C 1      | C 2     | C 3  |
|             |            | h                   | h       | h    |          | mg/L     | mg/L    | mg/L |
| 9/14/10     | 463        | 7.3                 | no data | 6.6  | 30       | 25.8     | no data | 25.6 |
| 9/15/10     | 467        | 7.1                 | 7.5     | 6.6  | 29.4     | 25.8     | 24.2    | 25.2 |
| 9/16/10     | 470        | 7.0                 | 7.0     | 6.7  | 29.2     | 26       | 25.8    | 24.7 |
| 9/17/10     | 473        | 8.1                 | 7.8     | 7.6  | 29.3     | 26.1     | 25.9    | 24.9 |
| 9/18/10     | 476        | 7.5                 | 7.6     | 8.0  | 29.5     | 24.8     | 26.1    | 25.9 |
| 9/19/10     | 480        | 7.7                 | 7.4     | 7.3  | 29.6     | 25.5     | 25.8    | 23.9 |
| 9/20/10     | 483        | 7.5                 | 7.1     | 7.0  | 29.1     | 25.5     | 25.8    | 24.5 |
| 9/22/10     | 486        | 15.4                | 14.4    | 13.8 | 29.1     | 25.7     | 25.9    | 24.7 |
| 9/23/10     | 490        | 8.0                 | 7.0     | 6.8  | 29.3     | 25.7     | 26.2    | 25.1 |
| 9/24/10     | 493        | 7.8                 | 7.5     | 6.8  | 29.5     | 25.8     | 26.2    | 25.1 |
| 9/25/10     | 496        | 7.6                 | 6.9     | 6.9  | 29       | 25.9     | 26.4    | 25.3 |
| 9/26/10     | 500        | 7.7                 | 8.0     | 6.9  | 29.4     | 25.3     | 25.6    | 24.4 |
| 11/19/10    | 491        | 7.7                 | 7.4     | 6.4  | 31.2     | 26.9     | 27.2    | 27.3 |
| 11/20/10    | 494        | 7.4                 | 7.1     | 7.1  | 31.1     | 27.5     | 27.9    | 26.9 |
| 11/21/10    | 497        | 7.7                 | 7.8     | 6.1  | 32.1     | 28.1     | 28.3    | 27.0 |
| 11/22/10    | 501        | 7.2                 | 6.8     | 6.9  | 31.0     | 27.9     | 28.2    | 27.6 |
| 11/23/10    | 504        | 7.6                 | 7.0     | 6.6  | 31.3     | 28.3     | 27.6    | 27.0 |

| Date    | PV<br>cum. | target 12-hour HRT  |      |      | [NO3-N]  |          |      |      |
|---------|------------|---------------------|------|------|----------|----------|------|------|
|         |            | HRT based on comp Q |      |      | influent | effluent |      |      |
|         |            | C1                  | C2   | C3   |          | C 1      | C 2  | C 3  |
|         |            | h                   | h    | h    |          | mg/L     | mg/L | mg/L |
| 4/27/10 | 119        | 11.2                | 11.7 | 10.1 | 28.3     | 17.3     | 15.3 | 17.0 |
| 4/28/10 | 121        | 11.2                | 10.9 | 10.1 | 28.2     | 18.9     | 16.1 | 17.6 |
| 4/29/10 | 123        | 11.5                | 11.0 | 10.5 | 28.1     | 19.2     | 16.6 | 17.4 |
| 4/30/10 | 126        | 10.3                | 10.1 | 9.8  | 28.5     | 19.3     | 18.6 | 18.1 |
| 5/1/10  | 128        | 10.6                | 10.7 | 10.4 | 27.7     | 19.3     | 17.5 | 17.5 |
| 5/3/10  | 132        | 10.9                | 10.7 | 10.1 | 27.6     | 19.7     | 17.5 | 17.9 |
| 5/4/10  | 135        | 11.1                | 10.1 | 10.4 | 28.4     | 20.4     | 19.6 | 18.9 |
| 5/5/10  | 137        | 11.3                | 10.0 | 9.3  | 28.5     | 19.7     | 19.1 | 19.1 |
| 5/6/10  | 139        | 11.7                | 10.4 | 9.7  | 28.7     | 19.9     | 18.8 | 20.0 |
| 5/7/10  | 141        | 11.7                | 10.7 | 9.9  | 27.9     | 19.1     | 19.2 | 19.5 |
| 5/8/10  | 144        | 10.9                | 10.4 | 9.9  | 28.8     | 19.1     | 17.7 | 18.1 |
| 5/9/10  | 146        | 10.9                | 10.7 | 10.0 | 28.6     | 20.1     | 18.8 | 19.0 |
| 5/10/10 | 148        | 11.1                | 10.8 | 10.2 | 30       | 20.5     | 19.8 | 19.2 |
| 5/11/10 | 150        | 11.7                | 11.3 | 11.9 | 29.4     | 19.3     | 18.9 | 17.3 |
| 5/12/10 | 152        | 12.1                | 12.0 | 10.4 | 29.4     | 17.2     | 16.7 | 13.8 |
| 5/13/10 | 155        | 10.6                | 10.9 | 9.8  | 29.5     | 16.5     | 14.1 | 15.4 |
| 5/14/10 | 157        | 10.8                | 11.3 | 10.0 | 30.1     | 17.9     | 13.7 | 14.4 |
| 5/16/10 | 161        | 10.9                | 10.4 | 10.6 | 30       | 20.3     | 18.0 | 16.1 |
| 5/17/10 | 164        | 11.1                | 10.8 | 10.5 | 29.3     | 19.5     | 17.0 | 14.1 |
| 5/18/10 | 166        | 11.1                | 10.9 | 9.3  | 29.6     | 19.3     | 17.3 | 16.5 |
| 5/19/10 | 168        | 11.8                | 11.4 | 11.3 | 29.6     | 18.8     | 16.7 | 14.4 |
| 5/20/10 | 170        | 10.5                | 9.3  | 9.8  | 34.5     | 18.5     | 15.4 | 14.3 |
| 5/21/10 | 173        | 11.7                | 10.7 | 10.1 | 30.9     | 18.7     | 17.3 | 14.5 |
| 5/22/10 | 175        | 11.3                | 10.7 | 10.2 | 30.3     | 18.3     | 15.1 | 14.5 |
| 5/23/10 | 177        | 11.4                | 10.9 | 16.2 | 30.6     | 19.1     | 15.7 | 14.7 |
| 5/24/10 | 179        | 11.6                | 11.3 | 10.6 | 30.2     | 18.7     | 16.9 | 9.9  |
| 5/25/10 | 181        | 11.5                | 10.9 | 9.5  | 30.2     | 18.0     | 14.7 | 14.2 |
| 5/26/10 | 184        | 10.7                | 11.0 | 9.6  | 30.5     | 18.8     | 14.4 | 14.3 |
| 5/27/10 | 186        | 12.0                | 11.1 | 9.7  | 30.5     | 18.6     | 14.5 | 14.5 |

| Date    | PV<br>cum. | target 12-hour HRT  |      |      | [NO3-N]  |          |      |         |
|---------|------------|---------------------|------|------|----------|----------|------|---------|
|         |            | HRT based on comp Q |      |      | influent | effluent |      |         |
|         |            | C1                  | C2   | C3   |          | C 1      | C 2  | C 3     |
|         |            | h                   | h    | h    |          | mg/L     | mg/L | mg/L    |
| 6/16/10 | 230        | 11.3                | 11.2 | 9.8  | 28.8     | 23.2     | 21.6 | 15.9    |
| 6/17/10 | 232        | 11.6                | 11.9 | 10.5 | 29.2     | 22.0     | 20.4 | no data |
| 6/18/10 | 235        | 11.2                | 11.2 | 9.3  | 29.6     | 21.5     | 20.7 | 16.0    |
| 6/19/10 | 237        | 11.0                | 11.6 | 9.4  | 28.9     | 21.6     | 20.6 | 16.4    |
| 6/20/10 | 239        | 11.2                | 10.1 | 10.2 | 29.0     | 22.0     | 21.8 | 16.3    |
| 6/21/10 | 242        | 11.0                | 10.1 | 9.7  | 29.1     | 21.3     | 21.3 | 15.9    |
| 7/3/10  | 261        | 11.1                | 9.2  | 10.1 | 30.7     | 20.7     | 22.9 | 13.0    |
| 7/4/10  | 264        | 12.1                | 10.1 | 10.8 | 30.9     | 20.6     | 22.2 | 10.8    |
| 7/5/10  | 266        | 12.5                | 10.6 | 10.8 | 30.9     | 21.0     | 21.1 | 11.5    |
| 7/6/10  | 268        | 11.8                | 11.2 | 10.8 | 31.4     | 22.3     | 22.2 | 14.8    |
| 7/7/10  | 270        | 12.0                | 11.1 | 10.8 | 30.7     | 22.9     | 22.6 | 17.7    |
| 7/8/10  | 272        | 11.9                | 11.2 | 10.9 | 30.6     | 20.4     | 19.8 | 15.6    |

| Date - time | PV<br>cum. | target 18-hour HRT  |      |      | [NO3-N]  |          |      |      |
|-------------|------------|---------------------|------|------|----------|----------|------|------|
|             |            | HRT based on comp Q |      |      | influent | effluent |      |      |
|             |            | C1                  | C2   | C3   |          | C 1      | C 2  | C 3  |
|             |            | h                   | h    | h    |          | mg/L     | mg/L | mg/L |
| 7/13/10     | 306        | 16.1                | 16.5 | 15.8 | 29.4     | 18.7     | 19.5 | 13.4 |
| 7/14 - 3AM  | 307        | 16.1                | 16.6 | 15.7 | 29.6     | 18.2     | 18.4 | 12.1 |
| 7/14 - 9PM  | 308        | 16.1                | 16.1 | 15.6 | 29.6     | 19.2     | 20.8 | 12.3 |
| 7/15/10     | 309        | 16.1                | 16.3 | 15.2 | 29.6     | 16.9     | 16.8 | 12.0 |
| 7/16/10     | 310        | 16.8                | 17.2 | 16.8 | 30.3     | 18.1     | 17.8 | 11.6 |
| 7/17 - 3AM  | 311        | 17.4                | 17.5 | 17.3 | 28.9     | 16.9     | 16.6 | 10.3 |
| 7/17 - 9PM  | 312        | 17.3                | 17.2 | 17.3 | 28.3     | 17.3     | 16.8 | 9.9  |
| 7/18/10     | 313        | 17.3                | 17.0 | 15.9 | 28.1     | 17.6     | 17.2 | 10.6 |
| 7/19/10     | 314        | 16.6                | 15.5 | 12.8 | 28.6     | 17.8     | 19.2 | 13.7 |



| Date - time   | PV<br>cum. | target 24-hour HRT  |         |      | [NO3-N]  |          |      |      |
|---------------|------------|---------------------|---------|------|----------|----------|------|------|
|               |            | HRT based on comp Q |         |      | influent | effluent |      |      |
|               |            | C1                  | C2      | C3   |          | C 1      | C 2  | C 3  |
|               |            | h                   | h       | h    |          | mg/L     | mg/L | mg/L |
| 7/20/10       | 316        | 22.3                | 19.1    | 18.8 | 29.0     | 24.5     | 16.4 | 12.3 |
| 7/21/10       | 317        | 23.4                | 20.5    | 20.2 | 28.0     | 14.7     | 15.9 | 8.5  |
| 7/22/10       | 318        | 22.9                | 20.5    | 19.3 | 27.9     | 15.2     | 16.5 | 8.9  |
| 7/23/10       | 319        | 23.3                | 20.5    | 20.1 | 28.9     | 15.0     | 16.5 | 8.7  |
| 7/24/10       | 320        | 23.2                | 19.0    | 20.3 | 28.7     | 15.4     | 16.4 | 8.8  |
| 7/25/10       | 322        | 23.4                | 21.3    | 20.4 | 28.8     | 15.7     | 16.5 | 9.1  |
| 7/26/10       | 323        | 23.2                | 22.3    | 20.5 | 29.3     | 15.9     | 16.6 | 9.1  |
| 7/27/10       | 324        | 22.2                | no data | 19.7 | 28.9     | 16.1     | 11.0 | 8.6  |
| 7/28/10       | 325        | 22.3                | 20.0    | 19.7 | 30.3     | 16.6     | 15.0 | 9.1  |
| 7/29/10       | 326        | 22.5                | 20.1    | 19.6 | 30.4     | 17.0     | 16.1 | 10.4 |
| 7/30/10       | 327        | 23.2                | 20.6    | 19.8 | 30.5     | 17.4     | 17.2 | 10.2 |
| 7/31 - 1:10PM | 328        | 23.3                | 21.3    | 20.2 | 30.0     | 17.6     | 17.9 | 9.4  |

| Date - time | Temp.<br>°C | HRT based on comp Q |      |      | [NO3-N]  |          |      |      |
|-------------|-------------|---------------------|------|------|----------|----------|------|------|
|             |             |                     |      |      | influent | effluent |      |      |
|             |             | C1                  | C2   | C3   |          | C 1      | C 2  | C 3  |
|             |             | h                   | h    | h    |          | mg/L     | mg/L | mg/L |
| 6/26/2011   | 10          | 12.9                | 13.3 | 14.4 | 29.2     | 23.2     | 23.0 | 22.2 |
| 6/27/2011   | 10          | 13.2                | 13.5 | 13.1 | 28.8     | 23.0     | 22.7 | 22.6 |
| 6/28/2011   | 10          | 13.2                | 12.6 | 10.1 | 28.5     | 23.2     | 23.7 | 24.3 |
| 6/29/2011   | 10          | 13.2                | 11.7 | 11.6 | 32.2     | 26.2     | 27.1 | 26.6 |
| 7/14/2011   | 10          | 13.0                | 11.7 | 12.8 | 31.7     | 24.5     | 25.3 | 24.3 |
| 7/15/2011   | 10          | 13.0                | 11.9 | 13.1 | 31.1     | 26.4     | 26.6 | 25.4 |
| 7/17/2011   | 10          | 13.2                | 12.3 | 12.6 | 31.8     | 27.5     | 27.6 | 26.3 |
| 7/18/2011   | 10          | 13.2                | 12.9 | 11.0 | 32.3     | 26.8     | 26.3 | 27.2 |

| Date - time | Temp.<br>°C | HRT based on comp Q |      |      | [NO3-N]  |          |      |      |
|-------------|-------------|---------------------|------|------|----------|----------|------|------|
|             |             |                     |      |      | influent | effluent |      |      |
|             |             | C1                  | C2   | C3   |          | C 1      | C 2  | C 3  |
|             |             | h                   | h    | h    |          | mg/L     | mg/L | mg/L |
| 7/19/2011   | 15          | 13.6                | 12.5 | 12.2 | 32.5     | 24.3     | 24.5 | 22.4 |
| 7/20/2011   | 15          | 13.7                | 12.8 | 12.4 | 33.1     | 25.0     | 24.7 | 24.0 |
| 7/21/2011   | 15          | 13.5                | 12.5 | 12.1 | 33.6     | 25.0     | 25.9 | 24.0 |
| 7/22/2011   | 15          | 13.8                | 12.7 | 12.2 | 32.6     | 23.9     | 25.2 | 25.0 |

| Date - time | Temp.<br>°C | HRT based on comp Q |      |      | [NO3-N]  |          |      |      |
|-------------|-------------|---------------------|------|------|----------|----------|------|------|
|             |             |                     |      |      | influent | effluent |      |      |
|             |             | C1                  | C2   | C3   |          | C 1      | C 2  | C 3  |
|             |             | h                   | h    | h    |          | mg/L     | mg/L | mg/L |
| 6/18/2011   | 20          | 12.7                | 13.6 | 12.7 | 29.1     | 14.6     | 12.8 | 13.1 |
| 6/19/2011   | 20          | 12.8                | 13.8 | 13.0 | 28.7     | 15.2     | 12.7 | 14.2 |
| 6/20/2011   | 20          | 12.7                | 13.8 | 13.4 | 28.0     | 15.7     | 14.3 | 14.7 |
| 6/21/2011   | 20          | 12.6                | 16.1 | 13.9 | 27.2     | 14.0     | 11.7 | 12.9 |
| 6/22/2011   | 20          | 12.4                | 13.5 | 13.8 | 26.7     | 13.2     | 12.2 | 12.1 |
| 6/23/2011   | 20          | 12.2                | 12.3 | 13.5 | 30.1     | 14.5     | 14.2 | 13.6 |
| 6/24/2011   | 20          | 12.8                | 12.9 | 14.0 | 28.7     | 13.6     | 12.7 | 10.7 |

## Appendix B: SAS code and statistics

### Code for analysis of removal difference between HRTs at room temperature

```

options formdlm = '-';
data influent;
  input hrt removal;
  cards; (insert data here)
proc means n mean stderr;
class hrt;
var removal;
title 'descriptive stats for hrt';
run;
proc glm;
  class hrt;
model removal = hrt;
lsmeans hrt/stderr;
title 'proc glm with anova and hrt means';
run;
proc glm;
title 'proc glm with estimates and MCP';
class hrt;
model removal= hrt;
lsmeans hrt/stderr;
/*diff 2-4 */
estimate 'Diff: 2-4' hrt -1 1 0 0 0 0;
/*4-8 */
estimate 'Diff: 4-8' hrt 0 -1 1 0 0 0;
/*8-12 */
estimate 'Diff: 8-12' hrt 0 0 -1 1 0 0;
/*12-18 */
estimate 'Diff: 12-18' hrt 0 0 0 -1 1 0;
/*18-24 */
estimate 'Diff: 18-24' hrt 0 0 0 0 -1 1;

```

### Code for analysis of removal difference with temperature change

```

options formdlm = '-';
data influent2;
input temp removal;
cards; (insert data here)
proc means n mean stderr;
class temp;
var removal;
title 'descriptive stats for tmp';
run;
proc glm;
class temp;
model removal = temp;
lsmeans hrt/stderr;
title 'proc glm with anova and tmp means';
run;
proc glm;
title 'proc glm with estimates and MCP';
class temp;
model removal= temp;
lsmeans temp/stderr;
/*diff 10-15 */
estimate 'Diff: 10-15' hrt -1 1 0;
/*15-20 */
estimate 'Diff: 15-20' hrt 0 -1 1;
/*10-20 */
estimate 'Diff: 10-20' hrt -1 0 1;
/*12-18 */

```

Least Squares Means  
Adjustment for Multiple Comparisons: Tukey-Kramer

| hrt | removal<br>LSMEAN | Standard<br>Error | Pr >  t | LSMEAN<br>Number |
|-----|-------------------|-------------------|---------|------------------|
| 2   | 0.12452782        | 0.00588390        | <.0001  | 1                |
| 4   | 0.07294669        | 0.00300523        | <.0001  | 2                |
| 8   | 0.06161010        | 0.00364127        | <.0001  | 3                |
| 12  | 0.12450897        | 0.00236941        | <.0001  | 4                |
| 18  | 0.09354358        | 0.00480419        | <.0001  | 5                |
| 24  | 0.08028518        | 0.00416055        | <.0001  | 6                |

Least Squares Means for effect hrt  
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: removal

| i/j | 1      | 2      | 3      | 4      | 5      | 6      |
|-----|--------|--------|--------|--------|--------|--------|
| 1   |        | <.0001 | <.0001 | 1.0000 | 0.0008 | <.0001 |
| 2   | <.0001 |        | 0.1591 | <.0001 | 0.0044 | 0.7088 |
| 3   | <.0001 | 0.1591 |        | <.0001 | <.0001 | 0.0106 |
| 4   | 1.0000 | <.0001 | <.0001 |        | <.0001 | <.0001 |
| 5   | 0.0008 | 0.0044 | <.0001 | <.0001 |        | 0.2973 |
| 6   | <.0001 | 0.7088 | 0.0106 | <.0001 | 0.2973 |        |

Least Squares Means  
Adjustment for Multiple Comparisons: Tukey-Kramer

| influent | removal<br>LSMEAN | Standard<br>Error | Pr >  t | LSMEAN<br>Number |
|----------|-------------------|-------------------|---------|------------------|
| 10       | 7.8238095         | 0.4035917         | <.0001  | 1                |
| 30       | 12.0057471        | 0.2804189         | <.0001  | 2                |
| 50       | 12.8936170        | 0.3815205         | <.0001  | 3                |

Least Squares Means for effect influent  
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: removal

| i/j | 1      | 2      | 3      |
|-----|--------|--------|--------|
| 1   |        | <.0001 | <.0001 |
| 2   | <.0001 |        | 0.1490 |
| 3   | <.0001 | 0.1490 |        |

| influent | removal<br>LSMEAN | 95% Confidence Limits |           |
|----------|-------------------|-----------------------|-----------|
| 10       | 7.823810          | 7.027212              | 8.620407  |
| 30       | 12.005747         | 11.452264             | 12.559230 |
| 50       | 12.893617         | 12.140583             | 13.646651 |